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Most significant biotechnological improvements on the production process of wine, beer and bread

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RESUM

Saccharomyces cerevisiae és el llevat més utilitzat en l'àmbit alimentari gràcies a la seva capacitat per dur a terme diferents tipus de fermentacions, com ara l'alcohòlica. Gràcies a aquest procés metabòlic ens podem beneficiar de productes com pa, vi i cervesa des de la prehistòria. En aquest treball s'ha fet una revisió bibliogràfica de les millores biotecnològiques més significatives per a l'optimització dels processos industrials de producció d'aquests aliments a partir de la modificació genètica i domesticació del *S. cerevisiae.* Per a la realització d'aquest treball de final de grau, s'ha utilitzat la base de dades d'articles científics PubMed, així com llibres impresos de la biblioteca de la Universitat de Girona i altres cerques a internet.

Pel que fa al vi, s'ha estudiat durant anys el mecanisme de resistència de *S. cerevisiae* a les altes concentracions de sulfits, els quals provoquen una alta toxicitat cel·lular. S'ha demostrat que les estratègies més efectives per afavorir aquesta resistència mitjançant la domesticació són utilitzar soques capaces de formar un complex no-tòxic amb acetaldehid, o bé que consumeixin sulfits per l'enzim sulfit reductasa i sulfitòlisi per glutatió , així com la sobreexpressió del gen SSU1 que codifica per una bomba de sulfit que destoxifica la cèl·lula. Per a la millora de la qualitat del vi s'utilitza l'enginyeria genètica proporcionant més cos i dolçor, per exemple amb la sobreproducció de glicerol. També, per aconseguir un gust més afruitat, cal que el llevat tingui la capacitat d'alliberar els monoterpens que conté el raïm, trencant l'enllaç glicosídic. Finalment, per evitar l'acidificació del vi, s'ha millorat el consum d'àcid màlic en la fermentació malolàctica.

En la producció de cervesa, els avenços biotecnològics han estat sobretot per enginyeria genètica i s'han centrat en la seva clarificació mitjançant la degradació de polímers lineals de glucosa pel propi *S. cerevisiae,* conferint-li la capacitat de secretar endoglucanases. Per a la producció de cervesa de baix nivell calòric, s'ha millorat la capacitat de degradació de les dextrines i altres hidrats de carboni derivats del midó. També s'ha pogut millorar el problema de l'acumulació de diacetil al final de la fermentació, el qual confereix a la cervesa gust dolç no desitjat, gràcies a soques transgèniques capaces de transformar el α-acetolactat en acetoïna, la qual no afecta el gust.

En referència a la producció de pa, s'han desenvolupat estratègies per a la producció de *S.cerevisiae* en grans quantitat de manera econòmica; per fer-ho, s'ha optimitzat el consum de melibiosa provinent de melasses, que s'utilitzen com a font de carboni. També s'ha estudiat utilitzar soques que prioritzin l'ús de la maltosa i així, incrementar la velocitat del procés de panificació. Per últim, s'ha estudiat com superar els efectes d'estres tèrmic produïts per la congelació de les soques *de S.cerevisiae* utilitzades en el procés de panificació, gràcies a la sobreexpressió de gens de protecció pel fred i també, d'aquaporines que ajuden a conservar la viabilitat cel·lular.

RESUMEN

Saccharomyces cerevisiae es la levadura más utilizada en el ámbito alimentario gracias a su capacidad para producir diferentes tipos de fermentaciones, como la alcohólica. Gracias a este proceso metabólico nos podemos beneficiar de productos como el pan, el vino y la cerveza desde la prehistoria. En este trabajo se ha hecho una revisión bibliográfica de los avances biotecnológicos más significativos para la optimización de los procesos industriales de producción de alimentos a partir de la modificación genética y domesticación de *S. cerevisiae.* Para la realización de este trabajo de final de grado, se ha utilizado la base de datos de artículos científicos PubMed, así como libros impresos de la biblioteca de la Universidad de Girona y otras búsquedas en internet.

En lo que respeta al vino, se ha estudiado durante años el mecanismo de resistencia de *S. cerevisiae* a las altas concentraciones de sulfitos, los cuales provocan una alta toxicidad celular. Se ha demostrado que las estrategias más efectivas para favorecer esta resistencia mediante la domesticación, son utilizar cepas capaces de formar un complejo no-tóxico con acetaldehído, o bien que consuman sulfitos por la enzima sulfito reductasa y sulfitólisis por glutatión, así como la sobreexpresión del gen SSU1 que codifica por una bomba de sulfito que detoxifica la célula. Para la mejora de la calidad del vino se utiliza ingeniería genética dando más cuerpo y dulzura, por ejemplo con la sobreproducción de glicerol. También, para conseguir un gusto más afrutado se necesita que la cepa tenga la capacidad de liberar los monoterpenos que contiene la uva, rompiendo el enlace glicosídico. Finalmente, para evitar la acidificación del vino, se ha mejorado el consumo de ácido málico en la fermentación maloláctica.

En la producción de la cerveza, las mejoras biotecnológicas se han producido sobre todo gracias a la ingeniería genética y se han centrado en su clarificación mediante la degradación de polímeros lineales de glucosa por la propia *S. cerevisiae,* confiriéndole la capacidad de secretar endoglucanasas. Para la producción de cerveza de bajo nivel calórico, se ha mejorado la capacidad de degradación de las dextrinas y otros hidratos de carbono derivados del almidón. También se ha podido mejorar el problema de la acumulación de diacetil al final de la fermentación, el cual le da a la cerveza gusto dulce no deseado, gracias a las cepas transgénicas capaces de transformar el α-acetolactato en acetoína, la cual no afecta al gusto.

Referente a la producción del pan, se han desarrollado estrategias para la producción de *S.cerevisiae* a gran escala de manera económica; para hacerlo, se ha optimizado el consumo de melibiosa proveniente de melazas, que se utilizan como fuente de carbono. También se ha estudiado utilizar cepas que prioricen el uso de la maltosa y así, incrementar la velocidad del proceso de la panificación. Por último, se ha estudiado como superar los efectos del estrés térmico producidos por la congelación de las cepas de *S. cerevisiae* utilizadas en el proceso de panificación, gracias a la sobreexpresión de genes de protección para el frio y también, de aquaporinas que ayudan a conservar la viabilidad celular.

ABSTRACT

Saccharomyces cerevisiae is the most used yeast in the alimentary field due to its ability to do different types of fermentation, such as the alcoholic one. Thanks to this metabolic process, we can benefit of many products such as bread, wine and beer, since the prehistory. This work is a bibliographic research about the most significant biotechnological improvements for the optimization of these industrial food processes based on both genetic modifications and domestication of *S. cerevisiae.* To carry out this final degree work, the scientific database PubMed and printed books from the library of the University of Girona and other internet searches were used.

Related to wine, the resistance mechanism of *S. cerevisiae* against high concentrations of sulphites, which cause cell toxicity, has been studied for years. It has been shown that the most effective ways to facilitate this resistance by domestication strategies are the use of strains capable of forming a non-toxic complex with acetaldehyde or consuming sulphites by the sulphite reductase enzyme, sulphitolysis by glutathione and, overexpression of the SSU1 gene, encoding a sulphite pump that detoxifies the cell. To improve wine quality, genetic engineering is used to give more body and sweetness, for example by glycerol overproduction. Also, for a more fruity taste a strain able to liberate the monoterpenes containing in grape, by breaking the glycosidic bond is required. Finally, to avoid wine acidification, malic acid consumption is improved in the malolactic fermentation.

In beer production, biotechnological improvements have been mainly achieved by genetic engineering, which has focused on the clarification by the degradation of linear polymers of glucose *S. cerevisiae*, conferring to it the ability to secrete endoglucanases. For the production of low calorie beer, it has been improved the *S.cerevisae's* ability to degradate dextrins and other carbohydrates derived from starch. The use of transgenic strains of *S.cerevisae*, able to to transform the α-acetolactate to acetoin, has overcome the problem of the accumulation of diacetyl at the end of fermentation, product that gives to the beer an undesired sweeteness; acetoin does not affect the taste.

Concerning to the production of bread, several strategies have been developed for producing large-scale *S.cerevisiae* biomass cheaply; for this purpose, the melibiose consumption from molasses has been optimized to use it as a carbon source. It has also been studied the use of strains that prioritize the utilization of maltose and, thus increase the speed of the baking process. Finally, it has been studied how to overcome the effects of thermal stress caused by the freezing *of S. cerevisiae* strains used in the baking process; the overexpression of genes for cold protection and also of aquaporins that helps to preserve cell viability have been successfully tested

1. INTRODUCTION

1.1. Brief fermentation history

During thousands of years, fermentation has been a very important process for food production and has been used as an effective way to preserve the quality and safety of beverages and foods. The earliest records appear in the Fertile Crescent (Middle East) and date back to 6000 BC; the preparation of these fermented foods and beverages was in an artisan way and without any knowledge of the role of the microorganisms involved (Blandino et al. 2003). It was in 1860 when fermentation process and its responsible, yeasts and bacteria, were firstly described by Pasteur (Fariña et al. 2012).

Yeasts are unicellular eukaryote fungi that belong to different taxonomic groups. Among these taxonomic groups, there is the genus *Saccharomyces,* which can be divided into two major groups: sensu stricto and sensu lato. The sensu stricto yeasts include *S. bayanus*, *S. cerevisiae*, *S. paradoxus* and *S. pastorianus* (syn. *S. carlsbergensis*) (Masneuf et al. 1998), it has been the most widely used by humans for preparing food and beverages, mainly by alcoholic fermentation, a process where the yeasts convert sugar into ethanol and carbon dioxide.
Bacteria, also often involved in fermentation process, are unicellular prokaryote microorganisms that produce different types of product fermentation such as alcohol, lactic acid, propionic acid, mixed acid or butyric acid (Brock et al. 2015).

Traditional fermented foods prepared from most common types of cereals (such as rice, wheat, corn or sorghum) are well known in many parts of the world. Some are utilized as colorants, spices, beverages and breakfast or light meal foods, while a few of them are used as main foods in the diet. In most of these products the fermentation is natural and involves mixed cultures of yeasts, bacteria and fungi. Some microorganisms may participate in parallel, while others act in a sequential manner with a changing dominant flora during the course of the fermentation. The common fermenting bacteria are genera like *Leuconostoc* spp., *Lactobacillus* spp., *Streptococcus* spp., *Pediococcus* spp., *Micrococcus spp.* and *Bacillus spp*. The fungi genera *Aspergillus spp.*, *Paecilomyces spp.*, *Cladosporium* spp., *Fusarium* spp., *Penicillium* spp. and *Trichothecium* spp. are frequently found in certain products. The common fermenting yeasts are species of *Saccharomyces spp.*, which perform normally in alcoholic fermentation (Blandino et al. 2003).

While a majority of yeasts have an aerobic metabolism, the majority of the *Saccharomyces* complex yeasts can survive without any oxygen by using the fermentation process. *Saccharomyces* sensu stricto yeasts species are basically specialized in carbohydrates. In absence of oxygen, they transform carbohydrates, such as glucose, into ethanol and CO_2 via the fermentation process. This can also occur in the presence of oxygen when there is a high concentration of carbohydrates because a ‘‘glucose repression’’ circuit represses the respiratory part in the presence of glucose. The occurrence of fermentation under aerobic conditions is sometimes referred to as the Crabtree effect and the yeasts exhibiting this peculiarity are referred to as Crabtree-positive yeasts (Sicard et al. 2011).

Thanks to fermentative processes, we can benefit from some products such as wine, bread and beer. It is believed that in ancient times, wine was made from wild or cultivated grapes and was mainly drunk and offered to gods during religious ceremonies or was used in medicine while beer was a popular drink. Beer was made from both emmer wheat (*Triticum dicoccum)* and barley (*Hordeum vulgare*), either separately or together, and with the same yeast from bread dough. Nowadays, both wine and beer are products used worldwide as a social drink and many companies have been trying to improve their organoleptic and chemical properties. Also, bread is one of the most old and eaten foodstuffs and it appears to be baked since Ancient Egypt (3000 a.C.). The first breads were made from coarsely milled emmer wheat (*Triticum dicoccum*) (Sicard et al. 2011). At present, this product is also worldwide known and the ingredients of dough change depending on the location of its production.

1.2. *Saccharomyces* sensu stricto

The *Saccharomyces* sensu stricto complex is composed by six sibling species, *S. cerevisiae*, *S. paradoxus, S. bayanus (S. bayanus var. bayanus), S. cariocanus, S. mikatae* and *S. kudriavzevii*. Interspecific hybridization has produced strains of industrial interest such as *S. pastorianus, S. monacensis* and *S. carlsbergensis. Saccharomyces uvarum* (*S. bayanus var. uvarum)* has also been considered hybrid yeast in this complex although recent DNA typing and sequencing analyses has suggested that it has become re-established as true species (Santos et al. 2007). The species *S. bayanus* is involved in lager beer fermentation, whereas *S. uvarum* has been isolated from wine and cider fermentations. Another specie in the sensu stricto group, named *S. paradoxus*, has been described as the main yeast in Croatian wines. The other three species in the sensu stricto complex, *S. cariocanus*, *S. mikatae* and *S. kudriavzevii* have been found in natural environments but never in fermentative environments (Belloch et al. 2008).

Sexual recombination is the most important process that generates genetic diversity in higher eukaryotes such as animals and plants. Similarly, in yeasts with a sexual life cycle (i. e *Saccharomyces* spp.), sexual reproduction can reshuffle the genomes of different yeast strains, thereby altering their characteristics and, potentially even lead to the evolution of new species such as *S.pastorianus,* a hybrid of *S.cerevisiae* and *S. eubayanus.*

In contrast to higher eukaryotes, yeasts like *Saccharomyces* spp. have also the ability to reproduce asexually, a much more prevalent way of reproduction than sexually, with only one meiotic cycle for every 1000 mitotic divisions on average. During these asexual reproductive cycles, spontaneous mutations, such as point mutations, indels, transposon insertions and recombination events can occur, leading to a higher genetic diversity (Steensels et al. 2014). Moreover, nucleotide changes are more frequent in intergenic regions than inside genes, as expected, because these intergenic regions have fewer restrictions for maintaining mutations than coding regions where many nucleotide changes would lead to non-functional amino acid changes (Herrero 2005).

Hybridizations between species of the sensu stricto complex, as well as variation of ploidy, have occurred many times and have allowed the genetic diversity of yeasts. The close

association of *S. cerevisiae* with human activities has led to the domestication of this specie, resulting in an organism that excels in its industrial task, but performs suboptimal in most other, more 'natural' environments (Steensels et al. 2014).

Differences in the specificity roles have led to a genetically diverse collection of individual strains that differ between them in single nucleotide polymorphisms (SNPs), strain-specific ORFs and localized variations in genomic copy number. These differences allow them to have very specific roles such as producing wine instead of beer (Borneman et al. 2011).

Saccharomyces cerevisiae is considered to be the agent of wine, ale beer, sake and palm fermentation, as well as the one of leavened bread (Sicard & Legras 2011). It is the referential yeast and the most investigated eukaryotic microorganism because it has been used in the production of food and alcoholic beverages for ages. It is a very attractive organism to work with since it is non-pathogenic, for its long history of application in the production of consumable products such as ethanol and it is also a good yeast for its well-established fermentation and process technology for large-scale production. Within the field of biotechnology, it is very useful due to its susceptibility to genetic modifications by recombinant DNA technology, which has been even further facilitated by the availability of its complete genome sequence published in 1996 (Marinoni et al. 1999; Ostergaard et al. 2000).

S. cerevisiae strains genetically cluster according to their ecological niches and type of fermentation rather than according to their geographical origins. For wine yeasts, 95% of strains isolated around the world belong to the same cluster, suggesting a unique origin of wine yeasts, followed by expansion of populations through human activities and geographic migrations (Sicard et al. 2011).

Related to beer and bread, the domesticated forms may have been achieved by natural and human selection, migration and mutation. To domesticate yeasts, genetic innovation, as well as restriction of gene flow between domesticated and natural forms is required. It is well accepted that inter-species hybridization and genome duplication have been important for the evolution of domesticated yeasts. The most famous interspecific hybridization events are probably the ones involved in yeast used for brewing lager beer (Sicard et al. 2011).

1.3. Wine making

Winemaking begins with the collection and crushing of grapes. For white wines, the grape juice is separated away from the skins and clarified via cold settling, filtration or centrifugation (Figure 1). In red wines the skins contain the red pigment and they are added to the fermentation, where will be dissolved together with tannins (Vogt et al. 1986). In both types of wine, the juice is then moved to a barrel or to a fermentation tank where the alcoholic fermentation is carried out by the indigenous yeasts of the juice. However, sulphur dioxide can be added to remove the indigenous yeast and then, selected starter cultures of *S. cerevisiae* and/or *S. ellipsoideus* (Morcillo et al. 2005; Mills et al. 2008), with tolerance to high doses of sulphites (Ramos et al. 2013), are added. Indigenous yeasts have lower alcohol tolerance than

commercial starter cultures and also can produce undesired compounds for the quality of wine (Brock et al. 2015).

In the case of red wines, after five days of fermentation are moved to another tank to continue the fermentation and to separate the sediment (precipitate of yeasts and cells). After that, the wine is stored at low temperature room to achieve the required maturity, the development of bouquet and further clarification. Finally, the wine is bottled and stored for final maturation, during a period of time ranging from months to years, or it also can be directly sold. In contrast, white wines are sold without maturation process (Brock et al. 2015).

Malolactic fermentation (MLF), in wine, is a secondary fermentation that usually occurs spontaneously at the end of alcoholic fermentation done by yeasts. This process determines the quality of red and some white wines and some sparkling wines and can be enhanced by a immobilized cell system (Genisheva et al. 2013). MLF is a biological process of wine deacidification in which the dicarboxylic L-malic acid (malate) is converted to the monocarboxylic L-lactic acid (lactate) and carbon dioxide. This process is normally carried out by lactic acid bacteria (LAB) originally isolated from wine. *Oenococcus oeni*, *Lactobacillus* spp. and *Pediococcus* spp. are the most commoly used bacteria, being *Oenococcus oeni* the preferred specie used to conduct MLF due to its acid tolerance and flavour profile produced (Liu 2002).

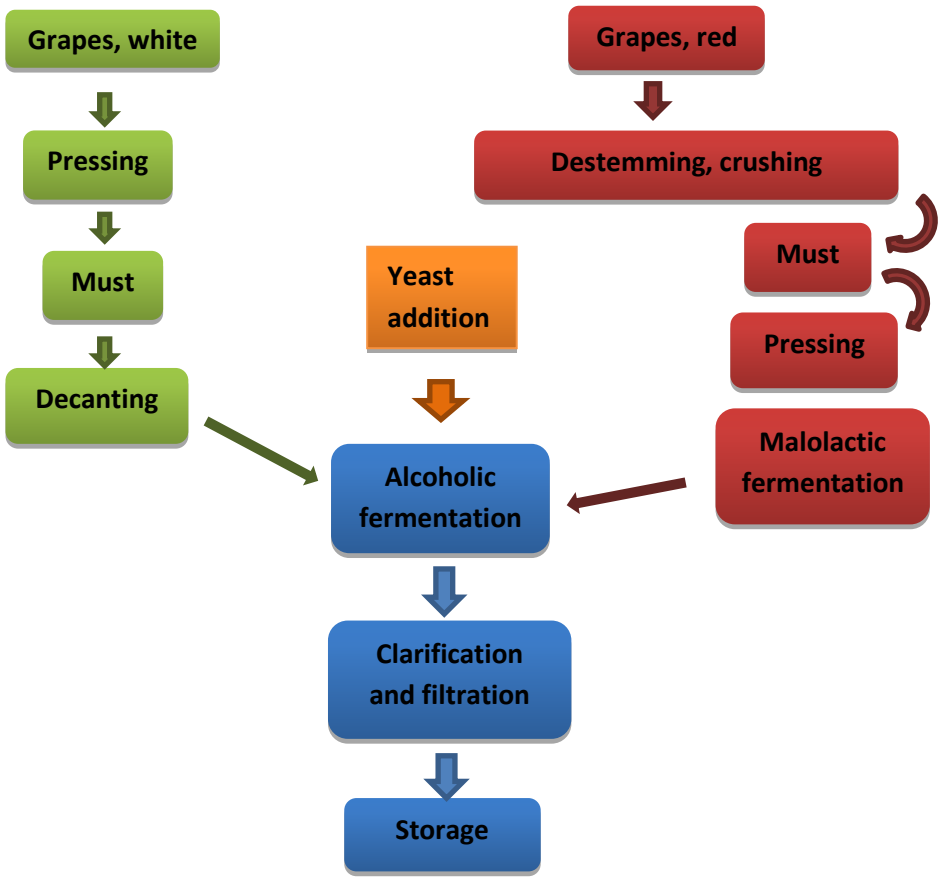

Figure 1. Wine making process flow chart. Adapted from Bamforth et al. (2007). Alimentos, fermentación y microorganismos, Ed Acribia.

1.4 Outline of Brewing

In this work, European-style beers will be taken as a reference because they are now produced worldwide, so they are the most common type of beer.

The simplest beer preparation involves incubating and extracting malt, which is ground up cereal grains (usually barley) with warm water. Sometimes the ground malt is mixed with other starchy materials and/or enzymes. The solution obtained is boiled with hops preparations and then clarified and cooled; finally, the liquid is fermented by added yeast (Briggs et al. 2004) (Figure 2).

Although all strains of *Saccharomyces* spp. produce ethanol as an end fermentation product, in practice the strains employed in the production of beers worldwide are classified into the categories of ale and lager yeasts:

- Ale yeasts, which are *Saccharomyces cerevisiae* strains, the most diverse yeast and the most isolated strains worldwide. These kinds of yeasts are often referred as "top-fermenting" yeasts because, in traditional open fermenters, they rise to the surface of the vessel, facilitating their collection by skimming, ready for repitching into the next fermentation.
- Lager yeasts ("bottom-fermenting" yeast), don't rise to the surface under any set of fermentation conditions; they have evolved, passing through iterations of *S. carlsbergensis* and *S. cerevisiae* lager type to the currently accepted name, *S. pastorianus*. Lager yeast is a more complex organism than ale yeast, and it has been proposed that its origin seems to be achieved by two separate steps involving the hybridization of *S. cerevisiae* with *S. bayanus* (Bokulich et al. 2013). Typical lager fermentation requires around 12 days to complete and therefore causes a 'bottle neck', holding up all the process. The design of breweries compensate for this 'bottle neck' by increasing fermenter size as well as the number of fermenters (Lodolo et al. 2008).

Ale yeasts differ from lager yeasts for their phenotypic and genomic characteristics. Among the major distinctive traits of these yeasts, there is the ability to ferment well at 20–25 °C. Lager strains generally cannot grow above 37 °C and ferment better at 8–10 °C (Ferreira et al. 2010).

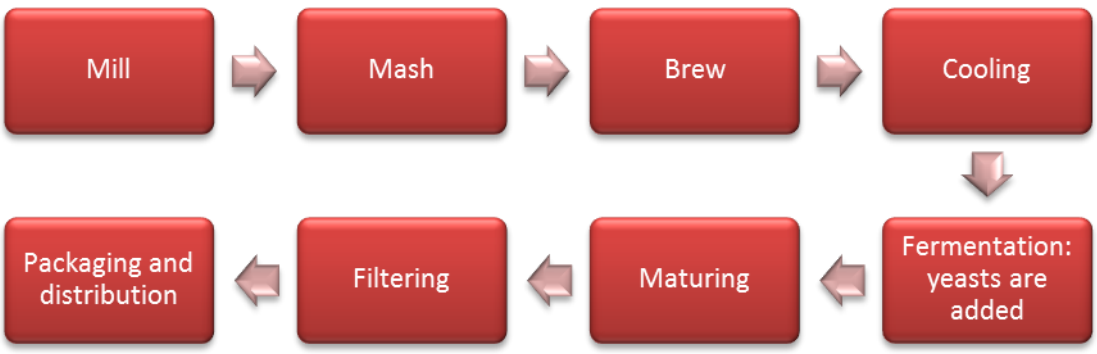

Figure 2. Brewing beer process flow chart. García-Garibay et al. (1999). Biotecnología alimentaria. Ed. Limusa.

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1.5 Baking Bread

Historically, sourdough was used as a leavening agent in bread production until it was replaced by baker's yeast (*Saccharomyces* spp.) in the 19th century. Baker's yeast enabled straight dough processes, which were adaptable to advanced automation in industrialized bakeries and, the use of sourdough fermentation was reduced to artisan and rye breads (De Vuyst and Michael Gänzle 2005). Sourdough is a mixture of mainly cereal flour and water, which is made metabolically active by a heterogeneous population of lactic acid bacteria (LAB) and yeasts, either by spontaneous fermentation or by fermentation initiated through the addition of a sourdough starter culture (De Vuyst et al. 2009). Lactobacilli, obligatory homofermentative and facultative or obligatory heterofermentative, are the typical sourdough LAB. *Lactobacillus sanfranciscensis*, *L. plantarum* and *L. brevis* are the most frequently isolated lactobacilli. Some strains, initially classified as *L. brevis*, were recently allotted to the new species and genera *L. pontis, Leuconostoc* spp. and *Enterococcus* spp. and are occasionally found or used in sourdough processes. Apart from LAB, several species of yeasts are found in sourdoughs; *Saccharomyces cerevisiae* is frequently present or added. The amount of *S. cerevisiae* may be overestimated due to the lack of reliable systems for identifying and classifying yeasts from this habitat. In particular *Saccharomyces exiguus*, *Candida krusei*, *Pichia norvegensis* and *Hansenula anomala* are yeasts associated with LAB in sourdoughs. The LAB:yeast ratio in sourdoughs is generally 100:1 (Gobbetti 1998).

Production of actual and standard bread starts with ground wheat flour or rye. Two enzymes, called proteases and amylases (α and β) and present in the wet dough, allow the release of simple sugars as maltose and sucrose from complex sugars (starch) present in the cereal seed; these sugars will be then used by yeast as feed for growth. Then, yeast's baker *Saccharomyces cerevisiae* is added. The initial aerobic conditions drive to a maximum production rate of CO_2 with a minimum production of alcohol. After this period, there is an anaerobic stage where the produced CO_2 raise the dough and produce the characteristic soft texture and flavour. During baking, yeasts are inactivated and alcohol and water of the dough, evaporate (Morcillo Ortega et al. 2005) (Figure 3).

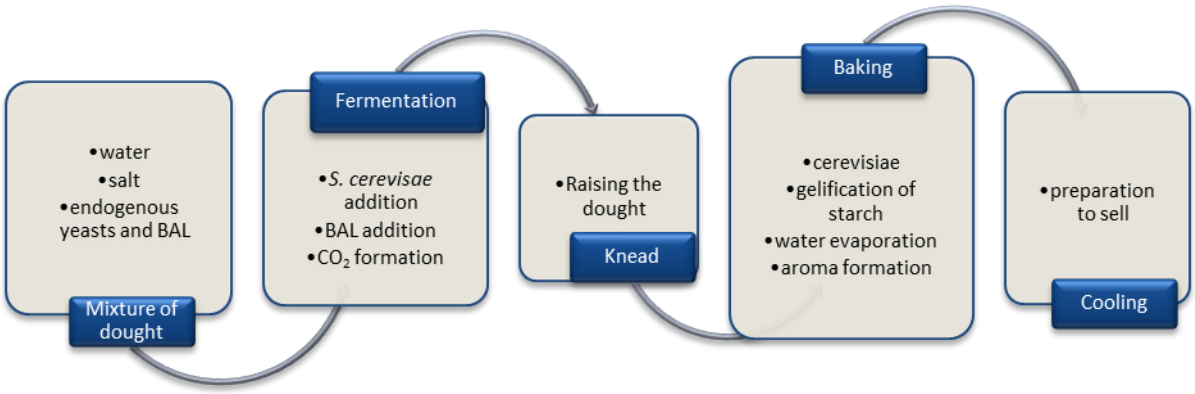

Figure 3. Baking bread process flow chart. Adapted from Bamforth et al. (2007). Alimentos, fermentación y microorganismos. Ed Acribia

2. OBJECTIVES

Saccharomyces cerevisiae is a yeast involved in fermentation, and therefore very useful in winemaking, baking and brewing since ancient times. Researches in molecular and cell biology have made possible the improvement of these industrial processes by increasing the production rate and the utilisation of different carbon sources by genetic engineering and domestication of the yeasts.

The aim of this work was to describe the most significant biotechnological improvements on the production process of wine, beer and bread. For this purpose, a bibliographic research about genetic engineering and domestication of *Saccharomyces cerevisiae* is presented in this study.

3. MATERIAL AND METHODS

This is a bibliographic work where many research articles and reviews from PubMed (www.ncbi.nlm.nih.gov/pubmed) were consulted. PubMed is a database that allows the free access to MEDLINE, a bibliographic database of citations and research articles about medicine and science in general.

Apart from the internet search, electronic books and printed books from the University of Girona's library have been used, as well as other scientific electronic journals databases have been referred .

4. RESULTS AND DISCUSSION

There are several ways to improve the metabolism of *Saccharomyces cerevisiae*; in this section, techniques like domestication or genetic engineering will be described and discussed. These techniques differ in the procedure by which they transform the strains. Domestication consists of picking the yeasts by human artificial selection considering their characteristics and, also crossing them with other different yeast to achieve the desired features. Genetic engineering consists of the direct transfer of DNA from one organism to another, the addition, or the deletion of genes using the newest technology.

In beer and bread, genetic engineering has played an important role to achieve improvements, while domestication has been really useful in wine production.

4.1 Wine

Some improvements on *S. cerevisiae* strains have been done in order to increase the tolerance to desiccation and viability of active dried yeast, on the grape sugar uptake and assimilation, on the increasing of ethanol tolerance, tolerance to sulphite, and to reduce foam formation (Pretorius 2000).

4.1.1 Resistance to sulphite

Sulphite is a reducing agent that prevents oxidation and bacterial spoilage and conventional winemakers have been adding it to wine for centuries to prolong its shelf life (Tan et al. 2010). A totally sulphite-free wine does not exist because sulphates are a natural product of fermentation and are present in wines without having been added (Berstein 2000). It is also a potentially toxic element but normal yeast produce it as an intermediate metabolite in the reductive sulphate assimilation pathway (Park et al. 2000). At pH values below 1.8, sulphite exists predominantly as free SO_2 and at pH values above 7.2, largely as SO_3^{2-}; at intermediate pH values, it exists in various proportions of bisulphite ion (HSO_3^-). The antimicrobial action of sulphite is greater at low pH values, fact that explains why the compound is particularly effective against yeasts which, in general, grow best at pH values in the range 3.0 – 5.0 (Pilkington et al. 1988).

Wine strains of *S. cerevisiae* with enhanced tolerance to sulphite, have been selected to use them as a preservative lately in the production process of wine (Park et al. 2000). At the gene expression level, sulphite represses genes involved in transcription, protein biosynthesis, and cell growth (Tan et al. 2010).
Cellular mechanisms that maintain redox homeostasis are crucial, because they provide a buffer against conditions that may perturb the redox environment of cells and/or induce oxidative stress (Tan et al. 2010). The mechanism of sulphite resistance of wine yeast has been studied by several groups. Stratford et al. (1986) reported that among the three forms of sulphite (SO_2, HSO_3^- and SO_3^{2-}), only the molecular form SO_2 was transported into *Saccharomyces cerevisiae* by simple diffusion. Hinze et al. (1985) reported that glyceralde-hyde-3-phosphate dehydrogenase was highly sensitive to sulphite and that sulphite uptake

resulted in a decrease in ATP concentration. Hara et al. (1980) reported that sulphite resistance was controlled by dominant polymeric genes and, finally, Casalone et al. (1992) isolated a sulphite-resistant mutant from a haploid laboratory strain and showed that a single dominant mutation was responsible for the sulphite resistance (Goto-Yamamoto et al. 1998).
Metabolic and genetic studies suggest that important means of protection against sulphite by *Saccharomyces* are: (1) formation of a non-toxic adduct with acetaldehyde, (2) sulphite consumption by sulphite reductase and sulphitolysis of glutathione and, (3) sulphite efflux (Park & Hwang 2008). It also has been reported for the first time the expression profiling changes by sulphite in *S. cerevisiae* using DNA microarray. The gene expression profiles suggested that genes involved in energy generation and acetaldehyde production, may account for the major acquired resistance to sulphite, and also, that sulphite represses genes involved in transcription, protein biosynthesis and cell growth (Park et al. 2008). Moreover, Pilkingkton et al. (1988) reported that sulphite, acetaldehyde and glycerol are produced in the same quantity by strains of *S. cerevisiae*. Furthermore, they showed that the production of acetaldehyde contributed significantly to the resistance to sulphite because of the correlation between ability of yeasts to grow in the presence of this compound.

Due to *S. cerevisiae* human domestication, the best strains with more potential than the others, are selected to do these investigations. So, glutathione is an ubiquitous thiol that maintains the intracellular redox state of *S. cerevisiae* by reducing cellular disulphide bonds and detoxifying damaging molecules such as xenobiotic and heavy metals (Outten et al. 2004). The ratio of reduced glutathione to oxidized glutathione within cells is often used as a measure of cellular toxicity (Pastore et al. 2001). Some researchers have shown that the GLR1 gene, encoding for yeast glutathione reductase (GR), is required to maintain a high intracellular reduced glutathione (GSH) to glutathione disulphide (GSSG) ratio (Trotter et al. 2003). The high abundance of glutathione (1–10mM) in cells and its low redox potential (-240mV) make the glutathione system an intracellular redox buffer in most cells. Moreover, mutants deleted for GLR1 are viable, over accumulate GSSG, and are hypersensitive to oxidants. Besides glutathione, cells have other redox-active molecules such as the thioredoxins and glutaredoxins that participate in oxidative stress defense (Tan et al. 2010).

In addition, SSU1 is a gene which encodes a plasma membrane sulphite pump involved in sulphite metabolism and detoxification. Mutations in SSU1 cause sensitivity, whereas overexpression confers heightened resistance (Goto-Yamamoto et al. 1998; Park et al. 2000). SSU1-R is an allele of SSU1 and its high rate of transcription, which may be due to the promoter sequence, was found to be responsible for the sulphite resistance (Goto-Yamamoto et al. 1998). SSU1-R lies on chromosome VIII and, in its upstream region, it contains four repeats of a 76-bp sequence. Yuasa et al. (2004) found that there was a complex relationship between the number of repeats and sulphite resistance. Moreover, Pérez-Ortín et al. (2002) found that sulphite-resistant strains presented a reciprocal translocation between chromosomes VIII and XVI as a nonhomologous recombination mediated by microhomology, which is very rare in wild-type strains. This mutation provokes the induction of the SSU1 transporter and increases the ability of yeast cells to expulse sulphite from the cytoplasm and provides them a better resistance (Sicard et al. 2011).

4.1.2 Body and sweetness in wine: increasing glycerol production

To improve the wine quality is interesting to make progress in the production of glycerol (produced while ethanol formation) which would result in a wine with more body and sweetness, so it is interesting to direct the carbon flux toward glycerol during ethanol formation in wine yeast (Ostergaard et al. 2000; Michnick et al. 1997; Nevoigt et al. 1996). The overproduction of glycerol at the expense of ethanol might represent an advantageous alternative for the development of beverages with low ethanol contents versus physical processes done by the time, which alter the organoleptic properties of the final product (Remize et al. 1999).

Glycerol, quantitatively the most important by-product of alcoholic fermentation, is synthesized by reduction of dihydroxyacetone phosphate to glycerol 3-phosphate (G3P), a reaction catalysed by NAD-dependent glycerol 3-phosphate dehydrogenase (GPDH) followed by dephosphorylation of G3P to glycerol by a specific glycerol 3-phosphatase (G3Pase) (Remize et al. 1999; Nevoigt et al. 1996). Its major role in fermentation is to maintain an intracellular equilibrium in redox balance by converting the excess NADH generated during biomass formation to NAD^+ (Remize et al. 1999). GPDH is encoded by GPD1 and GPD2, and the overexpression of GPD1 results in an increase of glycerol yield versus ethanol yield ratio (Ostergaard et al. 2000; Michnick et al. 1997; Remize et al. 1999; Nevoigt & Stahl 1996). Michnick et al. (1997) observed a significant reduction in final biomass in the strains that overexpressed GPD1 because glycerol fermentation *per se* results in net consumption of ATP.

4.1.3 Flavour improvement: increasing volatile compounds

Wine flavour is the result of the interaction among 500 volatile compounds. One of the most appreciated flavour is the fruitier, that depends on monoterpenes such as geraniol, linalool, and α-terpineol present in grapes. These compounds are found in the must, partly as free volatile forms and, partly as glycosidically bound nonvolatile precursors (as cellulose of the grape). This bound fraction needs to be hydrolysed to enhance wine flavour by adding exogenous enzymes extracted from filamentous fungus (Morcillo et al. 2005; Pérez-González et al. 1993).

A large proportion of these compounds are found as odorless diglycoside conjugates, which constitute a potential pool of aroma precursors after been hydrolysed. This enzymatic hydrolysis occurs in two steps. During the first step, and depending on the conjugate, the glycosidic linkage is cleaved by either a β-D-apiosidase, an α-L-arabinofuranosidase or an α-L-rhamnosidase and, the corresponding monoterpenyl-β-D-glucosides are released. In the second step, monoterpenes are liberated by the action of a β-D-glucosidase (Gunata et al. 1988; Manzanares et al. 2003).

Pérez-González et al. (1993) used the *Trichoderna longibrachiatum egll* gene, which encodes for a β-(1,4)-endoglucanase that cleave glycosidically bounds of cellulose, and transformed a *S. cerevisiae* strain to express this enzyme in the wine. The recombinant endoglucanolytic wine yeast secreted the fungal enzyme to the must, producing a wine with an increased fruity aroma.

Manzanares et al. (2003) reported that the use of several transgenic wine yeasts expressing a *Candida molischiana* β-D-glucosidase (Sánchez-Torres et al. 1998) and an *Aspergillus niger* α-L-arabinofuranosidase (Sánchez-Torres et al. 1996), were an useful tool to increase the free monoterpene content in wine. They also achieved the expression of genes encoding a *Trichoderma longibrachiatum* endoglucanase (Pérez-González et al. 1993) and different *Aspergillus nidulans* xylanases (Ganga et al. 1998; Ganga et al. 1999) in industrial wine yeast strains, which resulted in increased levels of some volatile compounds. In their study, they demonstrate that the gene THAA from *Aspergillus aculeatus* encodes for an α-L-rhamnosidase and it can be efficiently expressed in *S. cerevisiae*. The experiment was carried out with two strains: one expressing the *A. aculeatus* RHAA gene and the other expressing the *C. molischiana* gene BGLN, which codes for a β-D-glucosidase, because the enzymatic hydrolysis of grape rhamnoglucosides occurs in two steps and that the actions of an α-L-rhamnosidase and a β-D-glucosidase are needed to complete the process. The results showed an increase in linalool content due to the use of both strains together. Also, significant increases in α-terpineol and nerol levels were detected only in wines produced by both yeast strains compared to individual fermentations. These data indicated the biotechnological feasibility of the combination of wine yeast strains producing α-L-rhamnosidase and β-D-glucosidase to increase free monoterpenol content (Manzanares et al. 2003).

Another example of the improvement on the process of microvinification is the production of white wines with lower red colour, like the production of blush wines from red grape varieties. Pigments in red grape-derived musts are mainly anthocyanins which are composed of a flavylium ion glycosylated with a β-glycosidic bond. Breakage of this glycosidic bond liberates the corresponding anthocyanidin, that is spontaneously converted to a colourless pseudobase at pH levels within the range of those found in musts (Ueda et al. 1991). At high pH values blue/purple color is observed and at lower pH values red is observed (Brouillard 1982) . This enzymatic process is carried out by an anthocyanin-β-glucosidase (commonly termed as anthocyanase) and involves a decolourization of the juice. Potential applications of anthocyanase in winemaking include the prevention of sediments in the bottles during storage and obtaining free-run juice from red grape varieties in the production of white wines with lower red colour. Therefore, Sánchez-Torres et al. (1998) constructed a recombinant wine yeast strain expressing the *Candida molischiana* BGLN gene encoding for ananthocyanin β-glucosidase. Its corresponding protein, BGLN, and the recombinant strain used in that study decreased the colour of wine during microvinification experiment; however the physicochemical characteristics of the wines obtained with the recombinant wine yeast strain were indistinguishable from those obtained with the parental strain.

4.1.4 Improvement on the malolactic fermentation

Wine acidity plays an important role in determining wine quality and ensuring physiochemical and microbiological stability. In high-acid wines, the L-malic acid concentration is usually reduced through bacterial malolactic fermentation, while acidulation in low-acidity wines is usually done during final blending of the wine before bottling (Staden et al. 2005).

The ability to degrade malic acid by yeasts depends on the efficiency in the malate utilization system and malate transportation enzymes (i.e malolactic enzyme, malic enzyme, malate dehydrogenase or fumarase) (Seo et al. 2007).

It is known that strains of *Saccharomyces* spp. are inefficient metabolisers of extracellular L-malic acid due to their uptake system for L-malic acid (i.e. active import via a malate transporter) and a L-malic acid-converting enzyme (i. e. malolactic enzyme). Strains of *Saccharomyces* spp. lack the machinery for the active transport of L-malic acid and rely on rate-limiting simple diffusion for the uptake of extracellular L-malic acid. Labarre et al. (1996) described the first gene from *Leuconostoc oenos,* MLEA*,* coding for a malolactic enzyme. This gene was subcloned in *S. cerevisiae,* where the expression of MLEA conferred maolactic activity to this organism. However, an incomplete malolactic fermentation was shown, resulting in an insufficient malate uptake due to its lack of a malate transporter gene MAEL from *S. pombe* (Staden et al. 2005).

In conclusion, to achieve the ability to degrade L-malic acid during alcoholic fermentation with an industrial yeast like *S. cerevisiae*, the *S. pombe* malate transporter gene (MAEL) and the *O. oeni* malolactic enzyme gene (MLEA) have to be integrated in the same yeast genome (Staden et al. 2005).

4.2 Beer

S. cerevisiae beer strains have also developed specific mutations, achieved naturally or by genetic engineering, enabling them to have a better adaptation to malt brewing, the main carbon source of malt (Sicard & Legras 2011). Improvements related to the clarification of beer or the rate of production and adaptability to the medium will be explained in the next lines.

4.2.1 Improvement in beer clearance

One of the harder processes in brewing is to remove the lineal glucose polymers called β-glucans, which consist of short stretches of β-1,4-linked glucose moieties interrupted by single β-1,3-linkages (Bielecki & Galas 1991), that come from barley and impede beer filtration due to their high viscosity. *S. cerevisiae* produce a cell wall-bound exo and endo-β-1,3-glucanase (with β-1,3- and β-1,6-activites) but no glucanase with β-1,4-activity so it can't cleave the β-1,4 linkages of β-glucans and therefore commercial enzyme preparations would be necessary to carry out this process. As an alternative, a brewer's yeast (*S. cerevisiae)* with an heterologous gene encoding β-glucanase was designed (Van Rensburg et al. 1997; Ostergaard et al. 2000; Morcillo et al. 2005). To achieve the degradation of glucans, Van Rensburg et al. (1997) described the construction and over-expression of a cassette in *S. cerevisiae ,* consisting of genes encoding the three main types of glucanases: *S. cerevisiae* exo-β-1,3-glucanase gene (EXG1), that hydrolyze β-1,3-bonds next to β-1,3-linked glucose residues and β-1,6 linkages, together with the *Bacillus subtilis* endo-β-1,3-1,4-glucanase gene (BEG1) that only hydrolyzes β-1,4- linkages adjacent to β-1,3-linkages and with the B*utryvibrio fibrisolvens* endo-*β*-1,4-glucanase gene (END1) that hydrolyzes internal β-1,4-linkages next to β-1,4-linked glucose residues, with successful results.

Lu et al. (2009) reported the capability to secrete active endoglucanase and increase the sulphite accumulation in *S. cerevisiae,* by cloning the endo-β-1,4-glucanase gene EGL1 from *Trichoderma reesei* in a locus where a sulphite reductase was encoded in MET10 gene. Due to the partial elimination of the expression of MET10 gene, this recombinant yeast had the capability to hydrolyse glucans and also presented flavour stability due to the accumulation of sulphite.

Although filtration and centrifugation are good methods to clear beer, the most cost-effective method is flocculation. The flocculence is a process, consisting of contact and adhesion, where dispersed molecules or particles are held together by weak physical interactions, ultimately leading to phase separation by the formation of precipitates of larger size than colloidal ones (Jones et al. 2009). In addition, flocculation is a property ascribed to brewer's yeast and it would be interesting to introduce flocculence to a nonflocculent host strain with a genetic system that expresses its genes toward the end of fermentation. There are two distinct mechanisms of flocculation: the NewFlo phenotype, found in many brewer's yeast strains, and the Flo1 phenotype, containing the most flocculation genes and found mainly in flocculating laboratory strains (Ostergaard et al. 2000). FLO1 is the best studied gene and encodes for a cell surface protein that binds to neighboring cell wall mannoproteins during the flocculation (Teunissen & Steensma 1995); it has been optimized by some strategies such as cloning it under the transcriptional control of the ADH2 and HSP30 genes to enhance its expression. The ADH2 promoter is subjected to carbon catabolite repression and has been shown to be repressed during growth on glucose. Derepression of the ADH2 promoter coincides with transition to growth on ethanol. The HSP30 promoter is activated by several stress factors, including heat shock and sudden exposure to either ethanol or sorbate (Govender et al. 2008).

4.2.2 Starch utilization

Starch is composed by polysaccharides consisting of a large number of glucose units joined together primarily by α-1,4-glycosidic bonds and α-1,6-glycosidic bonds (You et al. 2013). This polysaccharide is produced by most green plants as an energy store. Depending on the plant, starch generally contains 20 to 25% amylose and 75 to 80% amylopectin by weight (Gurst 1984).

Many microorganisms, including *S. cerevisiae*, are not able to degrade starch since they do not produce starch decomposing enzymes such as α-amylase, β-amylase, pullulanase or isoamylase and glucoamylase. In order to utilize this carbon source, starch-descompositing enzymes or recombinant strains are added during brewing process (Ostergaard et al. 2000).

In beer there is about 3.3–3.4% of carbohydrates (75–80% dextrins, 20–30% monosaccharides and oligosaccharides and 5–8% pentosans) (Cortacero-Ramírez et al. 2003). Therefore, for production of low-calorie beer in the brewing industry, it is interesting to use a recombinant strain of *S. cerevisiae* that secretes a glucoamylase whereby the larger oligomers (dextrins) are decomposed. The species *Limpomyces kononenja¡oae* and *Saccharomycopsis fibuligera* are known to produce high amylase activities: *S.fibuligera* releases an extracellular glucoamylase with a high debranching activity and *L. kononenkoae* produces an extracellular α-amylase and a glucoamylase that are capable to produce a total starch hydrolysis. LKAI and LKA2 genes,

encoding α-amylases from *L. kononenkoae* and *S. fibuligera* respectively, have been cloned into *S. cerevisiae,* resulting in a high starch degradation (Knox et al. 2004; Moses et al. 2002; Eksteen et al. 2003).

The coexpression of the STA2 gene of *Saccharomyces diastaticus* encoding for a glucoamylase and an AMY1 of *Bacillus amyloliquefaciens* gene encoding for an α-amylase, was demonstrated to synergistically enhance starch degradation in *Saccharomyces cerevisiae* (Southgate et al. 1993). To achieve a major starch degradation, the PULA gene of *Klebsiella pneumoniae*, encoding for a pullulanase, was introduced into this transformant strain containing the *STA2* gene and the AMY1 gene and secreted using the yeast mating pheromone α-factor. This transformand yeast increased the starch hydrolysis rate compared to the parental strain and presented a complete assimilation (99%) (Janse et al. 1995).

4.2.3 Flavour improvement

The excessive residual saccharides, generated from the brewing process due to the slow and incomplete degradation of the sugar by brewing yeast, may result in beer with high caloric content and unusual flavour (Wang et al. 2012). The main problem on the production of beer is the accumulation of diacetyl at the end of fermentation, which confers sweetness. Diacetyl is a volatile compound and it is necessary a long period of lagering beer to volatilize it, but this process means a loss of efficiency because fermenters get blocked and any new production cannot start until this process is finished (Morcillo Ortega et al. 2005). The formation process of diacetyl starts spontaneously by a slow conversion of α-acetolactate to the unpleasant off-flavor compound diacetyl, which is enzymatically converted to acetoin and subsequentily to 2,3-butanediol (Ostergaard et al. 2000). The main strategy to overcome this problem is to avoid diacetyl formation and directly transform α-acetolactate to acetoin, which doesn't affect favour (Morcillo et al. 2005). Genetic modifications in *S. cerevisiae* by introducing a heterologous α-acetolactate decarboxylase (α-ALDC), which is able to decarboxylates α-acetolactate directly to acetoin without formation of diacetyl (Blomqvist et al. 1991), allowed the transformed strains to produce acetoin directly from α-acetolactate, fact that accelerated the brewing process by diminishing the time of lagering from weeks to hours.

Blomqvist et al. (1991) isolated the genes α-*ALD* and BUDA from *Klebsiella terrigena* and *Enterobacter aerogenes* respectively, which both code for an α-ALDC. These genes were cloned in *S. cerevisiae* and results showed that a single copy of this gene was able to reduce the diacetyl content with or without a short time lagering. Apart from the α-ALD gene of *E. aerogenes* and the BUDA of *K. terrigena*, the yeast strain used in this experiment contained no other foreign DNA, so it should be suitable for industrial production of beer.

Yamano et al. (1995) cloned the α-ALDC gene from *Acetobacter aceti subsp. xylinum* and it was integrated into the chromosome of a *S. cerevisiae ,* using the phosphoglycerate kinase (PGK) as the promoter, because it was suitable to express the ALDC gene in fermentation. Results showed that low productivity of total diacetyl was maintained stable during the successive brewing test.

4.3 Bread

A similar situation to that observed in beer industry occurs in baking industry: starch cannot easily be decomposed by *S. cerevisiae*, so it is advantageous to use certain recombinant strains that do not require α-amylase-enriched flour. Similar approaches to those used in brewing, have been performed in baking industry (see section 4.2.2).

4.3.2 Melibiose Utilization

Bread is one of the most consumed food in the word so, to produce it, a big amount of *S.cerevisae biomass* is required a . It has been pointed out that 430.000 tonnes (dry weight) are produced annually throughout the world. For obtaining this amount of biomass by a cheap way, the most frequently used carbon source is beet molasses, a waste product of the sugar manufacturing industry. Beet molasses contain sugars, sucrose, glucose and the trisaccharide raffinose in a 1-2% (w/w) (glucose-galactose-fructose) (Rønnow et al. 1999). Complete raffinose utilization requires both invertase (encoded by the SUC genes) and melibiase (encoded by the MEL genes) activities but expression of both enzymes occurs only under glucose repression; raffinose is therefore first metabolised when the glucose concentration is low (Rønnow et al. 1999; Vincent et al. 1999). The β-fructofuranosidase (invertase), encoded by SUC2 in *S. cerevisiae,* cleavages raffinose to fructose and the dissaccharide melibiose. Only fructose is metabolised since the α-galactosidic (α-1-6-linkage) bond between glucose and galactose in melibiose can't be cleaved by the baker's yeast due to its lack in one of the MEL1 to MEL11 genes coding for a α-galactosidase (Ostergaard et al. 2000; Vincent et al. 1999).

Many studies have been done to prove that is possible to transform any strain of *S. cerevisiae ,* to provide to it the ability to utilize raffinose and melibiose and, introduce this transgenic strain in markets where highly sugared doughs are used (Vincent et al. 1999). Atiyeh et al. (2003) demonstrated the ability of *S. cerevisiae* α-galactosidase-positive (MEL^+) transformant strain to completely hydrolyze raffinose, melibiose and sucrose to their monosaccharide components, and then to selectively convert glucose and galactose to ethanol and biomass. This study confirmed that the wild strain did not have α-galactosidase required for melibiose degradation.

Another example of melibiose utilisation is reported in the work of Gasent-Ramírez et al. (1995) which describes the characterisation of Mel^+ transformed baker's yeast strain, obtained by an one-step dominant-selection procedure, to construct recombinant *S. cerevisiae* without plasmid sequences. These strains were stable, increased the biomass/substrate yield in beet molasses about 8% and had growth rates similar to those of the untransformed control strains. The absence of bacterial DNA in the integrant structure would facilitate the commercial application of these procedures in the food industry.

4.3.1 Maltose Utilization

At the beginning of the bread-making process, yeast ferment the free sugars present in flour (mainly glucose, fructose, sucrose, maltose and glucofructans). The most preferred carbon sources for *S. cerevisiae* are glucose and fructose and when one of these sugars is present, the

enzymes required for the utilization of alternative carbon source are synthesized at low rates or not at all. This phenomenon is known as catabolite repression, and in this case it is called "glucose repression" and it is the source of the repression on the synthesis of maltose, galactose and sucrose-utilization enzymes (Klein et al. 1996). When these substrates are fermented, dough fermentation continues via the action of endogenous β-amylase and exogenous α-amylase, which release maltose from damaged starch granules. The faster maltose is consumed, the earlier the bread is done, so it would be ideal an initial consumption of maltose instead of free sugars. Although the amount of free sugars is low (1–2%), it is high enough for a catabolite repression (Ostergaard et al. 2000; Gancedo 1998; Randez-Gil et al. 1999). This causes a lag phase in CO_2 production where dough leavening is affected, the fermentative rate decrease and the time of baking increase (Randez-Gil et al. 1999).

Maltose used by *S. cerevisiae* is intracellularly degraded and it is required the presence of at least one of the five MAL gene loci, which contains at least one copy of three genes: MALR, coding for a regulatory protein, MALS, a maltase (α-D-glucopyranoside glucohydrolase) and MALT, a maltose permease. The gene MALR is expressed constitutively as a regulatory protein which binds near the MALS and MALT promoters mediating the induction of their transcription (Vanoni et al. 1989). Sucrose is hydrolyzed extracellularly by the action of invertase (SUC2) and differs with maltose in the induction of MAL genes: they need maltose as an inducer whereas sucrose doesn't (Ostergaard et al. 2000). Glucose repression, mediated by the protein MIG1, controls the expression of both the SUC2 gene and the MAL genes (Nehlin et al. 1990) and also the GAL genes (encoding enzymes responsible for galactose utilization) (Ostergaard et al. 2000). In the absence of glucose, MIG1 function is inhibited, directly or indirectly, by the Snf1p protein kinase, leading to derepression of gene expression (Celenza et al. 1989). Klein et al. (1996) disrupted the MIG1 gene in a laboratory strain and in an industrial polyploid strain and the effects of these disruptions on glucose control of both maltose and sucrose metabolism were investigated in batch culture. As a result, MIG1 disruption alleviated glucose repression of the MAL and SUC genes. However a pleiotropic effect was shown in the haploid and polyploidy strains that were used.

Another recently identified a protein, MIG2, similar to MIG1, also represses SUC2 expression in response to glucose. Additional deletion of MIG2 in a MIG1 mutant strain revealed further derepression of SUC2 expression (Lutfiyya et al. 1996). This demonstrates that deletion of MIG1 and MIG2 is very successful in the production process carried out by *S. cerevisiae* since glucose control is alleviated with respect to the sucrose metabolism.

Klein et al. (1996) improved maltose consumption by constitutive expression of the structural MAL genes. When MALT and MALS were overexpressed in the wild-type strain, maltose was utilized preferentially over glucose. Furthermore, the alleviated glucose control also revealed a shorter process time for bread production, which was further shortened because the dough may leaven even faster as a result of the increased specific growth rate (Ostergaard et al. 2000).

4.3.3. Freeze tolerance

Extreme environmental conditions arise during freezing, frozen storage, and thawing of bread dough, resulting in yeast cells with reduced viability and dough-leavening capacity. These effects have a great technological and economic impact because the yeast gassing rate is critical (Randez-Gil et al. 1999; Rodriguez-Vargas et al. 2002). Up to the date, the mechanism of freeze tolerance is not clear, even though many investigations are being done.

It has been shown that the adaptation of S. *cerevisiae* cells to downshift in temperature involves a control in some gene expression such as TIP1 gene (temperature-inducible protein) and two homologues: TIR1 and TIR2. The up-regulation of these genes is uncertain due to their lack of phenotype after a triple disruption (Δtip1, Δtir1, Δtir2) in a mutant (Kowalski et al. 1995). Rodriguez-Vargas et al. (2002) examined differential gene expression induced by cold or freeze shock stress. The results revealed that there were multiple genes induced by cold and freeze and most of them were repressed. This reflects the decreased rate of growth associated with a shift to low temperatures as a general stress response. Nine genes were identified in their study: TIP1 and ERG10 (gene for acetoacetyl-CoA thiolase) were two of them and specifically, it was found that high expression of ERG10 reduced freeze sensivity in S. cerevisiae, suggesting that overexpression of cold-induced genes could be a useful tool to improve the adaptive response to these stress conditions in industrial strains.

Tanghe et al. (2002) revealed a correlation between freeze resistance and the aquaporin genes AQY1 and AQY2. Aquaporins belong to the major intrinsic protein (MIP) family of membrane proteins. They are involved in the transport of water and/or small neutral solutes such as glycerol, where AQY1 and AQY2 are water channels (Carbrey et al. 2001). In the study of Tanghe et al. (2002), these genes were disrupted in a laboratory strain and the cells became more sensitive to freezing, but when these genes were overexpressed, the freezing tolerance was improved. Aquaporin overexpression also improved maintenance of the viability of industrial yeast strains in small doughs stored frozen and, furthermore, a transformant strain with overexpressed aquaporins could be selected based on its improved freeze-thaw resistance without the need for a selectable marker gene, which is an interesting feature to develop for application in the food industry.

As it has been shown in this work, different genetic engineering improvements and domestications to get a better industrial food processes have been done or are currently being done. In beer, wine and bread production, *Saccharomyces cerevisiae* plays and important role in fermentation but it would not be so significant without others microorganisms genes. Science is in continuous progress, enabling us to get the best abilities of microorganisms while preserving environment and providing us new ways to improve the quality of our food and beverages.

5. CONCLUSIONS

Wine production

- The principal protections against sulphites are the formation of a non-toxic adduct with acetaldehyde, overproduction of glutathione and overexpression of SSU1, a gene which encodes a plasma membrane sulphite pump involved in sulphite metabolism and detoxification; overexpression of glutathione and SSU1 confers heightened resistance.
- The overproduction of glycerol leads to a major body and sweetness and low ethanol content in wine. The overexpression of GPDH, a glycerol 3-phosphate dehydrogenase, results in an increase of glycerol yield versus ethanol ratio.
- The fruitier flavour is given mainly by geaniol, linalool and α-terpineol, but they have to be hydrolysed by exogenous β-glucosidase, which enables *S. cerevisiae* to cleave glycosidically bounds.
- An exogenous malate transporter gene and a malolactic enzyme gene could be integrated in the *S. cerevisiae* genome to decrease the acidity of wine.

Beer production

- The best ways to clarify beer using *S. cerevisiae* strains are: to remove β-glucans with an exogenous β-glucanase and enhance the expression of the flocculence gene *FLO1*.
- The introduction of a glucoamylase, α-amylase or/and a pullulase gene into *S. cerevisiae* strain genome increases starch degradation and produces low-calorie beer.
- To overcome the source of sweetness, an unpleasant flavour in beer, the main strategy is to avoid diacetyl by introducing a heterologous α-acetolactate decarboxylase to a *S. cerevisiae* strains.

Bread production

- To produce a large amount of baker yeast, *S. cerevisiae*, by a cheap way, beet molasses are used as a carbon source. For the total use of molasses, it is necessary to degrade raffinose to melibiose, using an invertase (coded by SUC genes) and a melibiase (coded by MEL genes). *S. cerevisiae* lacks of MEL genes, so it is interesting to transform the yeast to a MEL positive strain, which would increase the biomass/substrate yield.
- The genes SUC2 (coding for an invertase) and MAL (coding for a maltase, maltose permease and a regulatory protein) are repressed by MIG1 in presence of glucose. To improve the production of bread, is interesting to disrupt MIG1, enabling SUC2 and MAL to degrade sucrose and maltose, would accelerate the baking process.
- To overcome the decreased rate of growth, gassing, viability and dought-leavening capacity of *S. cerevisiae* cells caused by cold temperatures, overexpression of cold-induced genes as ERG10 and of aquaporins such as AQY1, could be an useful tool to improve the adaptative response to stress.

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