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REVIEW ARTICLE

Impact of the genetic improvement of fermenting yeasts on the organoleptic properties of beer

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Abstract

The brewing industry has experienced a significant boom in recent years through the emergence of, on the one hand, craft breweries that produce beers with unique organoleptic characteristics, and, on the other hand, the brewing of a significant number of beers using hybridized or genetically modified microorganisms with the aim of improving both the brewing processes and the final products. This review covers the influence from yeast strains on the organoleptic properties of the final beers and also the main hybridization and genetic modification methods applied to such yeast strains with the aim of improving the sensory characteristics of the product obtained and/or the brewing process. Different approaches to the phenotypic modification of the yeasts used in beer brewing have arisen in recent years. These are dealt with in this work, with special emphasis on the methodology followed as well as on the effects of the same on the brewing process and/or on the final product.

Keywords Beer · Brewing · Yeast strain · Hybridization · Molecular genetics

Introduction

Brewing beer is one of the most ancient biotechnological processes, along with the production of bread and wine, performed by humans. It probably dates back to the time when humans evolved from hunter/gatherers to sedentary farmers (Late Neolithic–Early Bronze Age). However, the first written records where beer making is specifically mentioned date back to the Mesopotamian culture around 2800 BC. Likewise, in the Babylonian and Egyptian cultures, numerous material evidences have been found that support the production of beer by these civilizations. Likewise, evidence has been found pointing at the malting of barley as part of the beer production methods used by these ancient societies [1, 2].

In Europe, during the fourteenth century, the brewing process gradually transformed into a "pre-industrial" process, where both the scale of production and the purpose of the brewing process changed radically. This transformation took place mainly in the monasteries throughout Central Europe [1]. Along with these first attempts to "industrialize" the brewing process, two fundamental events in the evolution of the brewing process occurred: the introduction of hops as an important part of the brewing process and the implementation of the first regulations on the brewing process itself [1].

The discovery of the microorganisms (yeasts) responsible for the fermentation processes by Louis Pasteur in the second half of the nineteenth century, as well as the studies by Emil Christian Hansen on the method to isolate and individually propagate yeasts were both extremely important milestones toward what brewing processes are today [3].

Yeasts are generally unicellular eukaryotic organisms that belong to the fungal kingdom and unlike plant cells, they do not need sunlight to metabolize. They are responsible for the anaerobic fermentation processes that transforms sugars into alcohol and carbon dioxide [4, 5]. Two species have been traditionally used for brewing, *Saccharomyces*

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cerevisiae (ale fermentation) and *Saccharomyces pastorianus* (lager fermentation), which in addition to carrying out the fermentation, contribute with other by-products (such as esters, lactones, thiols, or phenolic compounds) to the organoleptic characteristics of each type of beer [6–8]. In addition to conferring organoleptic traits, yeasts are involved in a number of processes that can be decisive for the quality of the final product, such as the clarity of the beer and the stability of its foam [8–10].

Since each yeast strain confers to the final product a number of particular characteristics, the choice of the yeast strain is a crucial aspect when it comes to determining which type of beer will result from the whole procedure. However, just as each strain contributes with its "positive" characteristics to the final product, it may also add its non-desired features (technical difficulties, turbidity, unstable foam, organoleptic flaws, etc.). Hence, the ideal procedure would allow us to select the attributes from each strain that we consider of interest, or otherwise to discard those that are less desirable, to "generate" a new strain with the specific qualities that best suit the final product we are trying to obtain. With this in mind, different approaches to the phenotypic modification of the yeasts used in beer brewing have emerged in recent years. As can be seen in Fig. 1, the number of works dedicated to genetic improvement or the use of hybrids in foods, beverages or beers has significantly increased in these last 10 years. In the case of beer, most of them are discussed in this paper.

Methodological aspects

Hybridization

One of the first procedures that have been used to achieve this objective consists in the breeding of hybrids from different strains, so that by combining the specific properties from each hybridized strain, the desired qualities would be present in the resulting hybrids. Hybridization, both intraspecific and interspecific, is not an easy task and despite the low sexual fertility of industrial yeasts, which is a necessary condition to successfully complete their hybridization, significant progress has been made in obtaining stable *S. cerevisiae ale/S. cerevisiae sake* hybrids, whose final products contain more ethanol and esters than their respective individual strains. Some examples of this method were reported by certain authors [11, 12]. Also *S. cerevisiae* ale and cold-tolerant *S. bayanus* hybrids allow lower fermentation temperatures than the initial *S. cerevisiae* ale strain [11, 13]. Another example is the *S. cerevisiae* ale/wine hybrid *S. eubayanus*, which results in a yeast strain that can be used to brew beers with much higher aromatic contents than those obtained from its parent strains. Finally, other examples can be the use of S. cerevisiae hydrids that can remain in bottled beer for more than a year and are employed to eliminate/reduce the accumulation of staling aldehydes [11, 14, 15]

The main problem linked to the breeding of hybrids is the low proportion of these that contain the traits that are sought for from the original strains, which makes of it a rather random process [16]. Thanks to the considerable

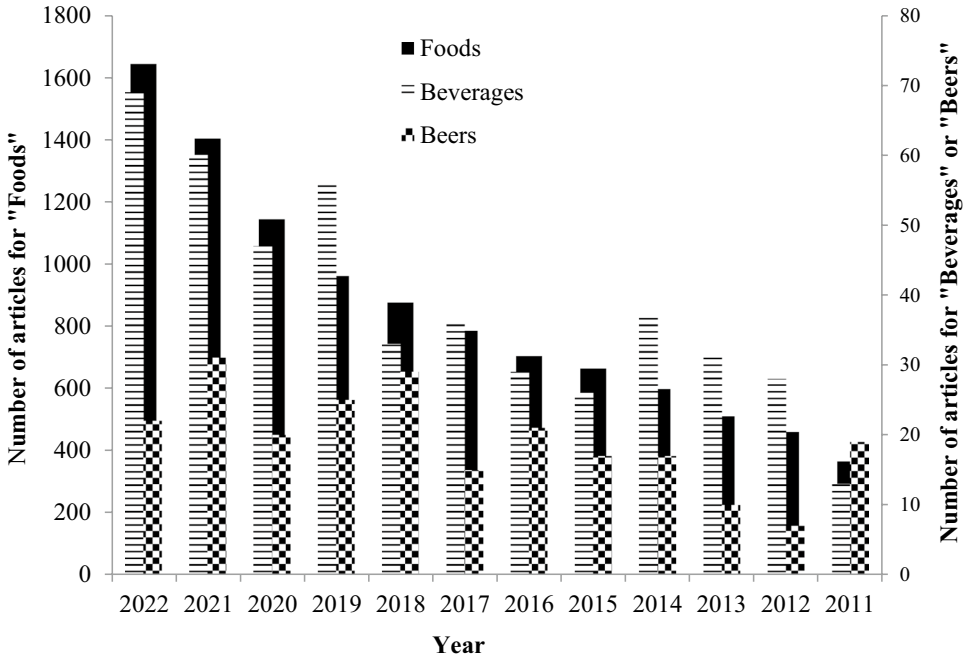

Fig.1 Number of works dedicated to genetic improvement or the use of hybrids in foods, beverages or beers (Scopus)

advances achieved in recent years regarding the development of biotechnological processes, a new avenue has opened up to tackle this quest for the "ideal" phenotype. Current molecular genetics allows us, through relatively simple laboratory processes, to specifically add or eliminate the genes that are associated to certain phenotypes, so that the desired strain can be obtained in an almost "ad hoc" manner [16].

Random mutagenesis

Parallel to these genetic modification technologies, a number of experiments consisting of the mutation of yeast genes through UV light mutagenesis or through chemical mutagens [16–18] have also been implemented. In these cases, the mutations are made randomly and entail a complex and laborious process of selecting those mutants that exhibit the desired traits [19]. The advantage of molecular genetics lies in the fact that it can obtain hybrids more rapidly than other traditional methods, nevertheless and due to the random nature of the mutations, the mutated hybrids that exhibit the desired traits of interest, may also contain other undesired mutations that could result in technical or sensory inconvenience [20].

Molecular genetics

Numerous methodologies have been applied to genetic transformations. Nevertheless, most of them can be classified into one of the following two groups: recombinant DNA engineering techniques or metabolic engineering processes [16].

Standard molecular genetics techniques require the desired premises to be established previously to perform any genetic modification. Therefore, when we pursue a certain trait (phenotype), we need to precisely determine which gene or genes are associated with that trait (genotype). Once they have been identified, we can address their modification and, for this purpose, a number of genome modification processes based on the use of recombinant DNA technology can be implemented.

Recombinant DNA techniques

This set of techniques can be further categorized into two subgroups; recombinant vector or yeast genome insertion technologies [16].

There are a large number of yeast expression vectors that can be used to produce recombinant proteins. They all contain common elements in terms of function (yeast replication origins, specific promoters to start the transcriptions, sequences containing antibiotic selections…) as well as a multiple cloning site where the gene sequence to be expressed can be inserted [21]. All these vectors contain specific genes that confer resistance to various antibiotics for the selection of those transformants contained in the construct. Therefore, for the selection of the transformed strains, it is essential to include the selection antibiotic, as well as to prevent the loss of this in subsequent processes due to the mosaic effect. All this implies that the development of these strains carrying these constructs is more oriented to the investigation of their qualities at a laboratory level than to their use as industrial strains. Once the construct is completed, it is introduced into the host strain (transformation process) and the transcription and further translation of the recombinant protein by the modified strain initiate. Finally, whether the modification introduced into the yeast produces the expected phenotypic change remains to be evaluated. This technique is very useful when the genes associated to a particular trait are unique or rare. If the trait that is being sought is known to be produced by the joint action of a large number of genes, we can rely on different DNA assembly technologies [22]. By means of these techniques, we can simultaneously express multiple recombinant proteins in yeast by generating constructs that contain the sequences of the different genes to be expressed in tandem [23, 24]. In any case, these techniques do not imply the integration of these vectors into the yeast genome, but remain as independent self-replicating structures. This fact can lead, if very strict selection conditions are not maintained, to the loss of the plasmid due to stochastic segregation [25]. To prevent this negative effect to occur, the constructs can be integrated into the yeast genome, which will imply that the modified strain will replicate the inserted gene/genes with the rest of its genome. This process is carried out by homologous recombination, according to which a fragment of the yeast genome is replaced by a fragment containing that same sequence (hence it is called homologous) plus all the genes and control elements that we need for the expression of the proteins of interest. Likewise, it can be used to break the reading pattern of a given yeast native gene, thus creating a "knockout" mutant yeast for that gene and, therefore, for its associated trait [26, 27]. However, homologous recombination requires a complex screening process to determine those clones that have correctly integrated the heterologous genotype into their sequence. In recent years, a new homologous recombination technique known as CRISPR/Cas9 (Emmanuelle Charpentier and Jenifer A. Doudna, Chemistry Nobel Prizes in 2020) has been developed, which allows targeted gene editing with a high integration efficiency. This technique permits a smaller homologous DNA and a much simpler and faster mutant selection [28, 29]. Using this method, up to 15 DNA fragments (all belonging to the β-carotene metabolic pathway) have been integrated at 3 separate loci [30] or similarly 10 DNA segments (from muconic acid metabolism) have been integrated at 3 different loci allowing the simultaneous silencing of unwanted genes through a break in the reading pattern [31].

Targeted metabolic engineering

One of the main problems associated with the previously mentioned techniques is the low probability of the resulting phenotype to avoid the collateral effects caused by the introduction of an exogenous gene. As an alternative to these methods, targeted metabolic engineering allows specific modifications of the genetic information (such as overexpression or gene silencing among other possibilities) and its corresponding phenotype [16]. These techniques require an exhaustive knowledge of the metabolic pathways involved in the processes we want to alter, so that we can intervene, almost surgically, by including genes that produce enzymes that modify those pathways, overexpressing endogenous genes to enhance certain metabolic pathways or silencing others that inhibit their associated pathways. These techniques have always been used in brewer's yeasts with the aim of improving fundamental aspects of the brewing process, for example, to:

- Intensify the production; seeking to accelerate the fermentation and maturation processes [32]; to produce yeasts that use starch as a source of sugars for fermentation [33]; or strains that improve the flocculation process [34].

- Improve beer quality, either by trying to obtain strains that modify the metabolism of active sensory compounds (mainly oriented to the elimination, as far as possible, of diacetyl) [35, 36]; strains oriented to improve foam stability and quality [37]; or the brightness of beer [38].

Table 1 summarizes some of the advantages and disadvantages of the different genetic improvement methodologies applied to beer.

Biotechnological applications oriented to beer brewing

Creating and choosing new hybrids

It has been discussed throughout the text that the choice of the yeast strain and its associated phenotypic characteristics in the beer fermentation process is fundamental for beer production, since small genetic differences, with their corresponding phenotypic differences, will give rise to different types of beer. An example of this is “weizen” German beers. As demonstrated by Mukai et al. [39], the ale yeasts used to produce these beers differ from others in the *PAD1* and *FDC1* genes. By inducing a single nucleotide polymorphism in these genes, the resulting variation of its enzymatic activity enables ferulic acid decarboxylation. Consequently, these strains give a clove-like aroma and are, therefore, only used either for the production of wine or of this peculiar German beer.

Genetic differences between strains may concern just a few genes, as in the previous case, or they may be greater and give rise to different species. This has been very important in the development of beer production processes since, although *Saccharomyces cerevisiae* (ale) was originally the yeast used, nowadays lager type yeasts are widely used. As already mentioned, this group includes *Saccharomyces pastorianus*, which was born as a natural hybrid from *Saccharomyces cerevisiae* and *Saccharomyces eubayanus*. This biological fact has resulted in these two types of yeast (ale and lager) producing beers with different organoleptic characteristics and presenting different behavior with respect to certain factors such as, for instance, fermentation temperature [16, 40]. At present and because of this frequent use of lager yeast, some research efforts have been made to produce new targeted hybrids whose offspring present certain phenotypic characteristics. An example of this is the study by Nikulin et al. [41] who experimented with the production of *Saccharomyces cerevisiae* hybrids using *S. arboricola*, *S. mikatae*, and *S. uvarum*. The hybrids that were obtained achieved better fermentation processes than their parental species under regular lager fermentation conditions. In addition, they produced more long-chain alcohols and esters, which resulted in the brewing of more aromatic beers. Breeding and using these artificial hybrids for the production of lager beers is, therefore, a suitable alternative to the use of *S. pastorianus*. In a related study, new strains of *S. pastorianus* were produced by spore fusion. Thus, after sequencing the genome of different *S. pastorianus* yeast strains, it was found that all the lager yeasts were genotypically grouped into two types: "Saaz" and "Frohberg", which explained the limited diversity of organoleptic profiles of lager beers compared to ale ones or wine. On these grounds, different strains of *S. cerevisiae* and *S. eubayanus* were crossed in that study with the aim of obtaining new strains of the "Saaz" and "Frohberg" types that would not only be genotypically different, but would also exhibit different phenotypical characteristics. As a result, totally new hybrids were obtained that showed, at laboratory and pilot scale, the ability to grow properly, to carry out fermentation and, above all, to produce new aromas and flavors. It could be concluded that these new hybrids could well be used at industrial scale to brew new lager beers [14, 42].

Random mutagenesis by UV radiation

Genetic differences are undoubtedly a decisive factor in terms of the final result. That is the reason why breweries and beer brands normally preserve their yeast strains and carry out an exhaustive controls to ensure that their beers do not experience any variations and that the same product is always delivered to consumers [43].

Table 1 Advantages and disadvantages of the different genetic improvement methodologies applied to beer

Technique	Advantages	Disadvantages
Hybridization	No complex molecular techniques are necessary	Low success to complete the hybridization due to the low sexual fertility of industrial yeasts It is a rather random process
Molecular genetics: recombinant DNA engineering techniques	The desired strain can be obtained in an almost "ad hoc" manner It can obtain hybrids more rapidly than other traditional methods Technique very useful when the genes associated to a particular trait are unique or rare CRISPR/Cas9 homologous recombination technique, simpler and faster mutant selection process	Low probability of the resulting phenotype to avoid the collateral effects caused by the introduction of an exogenous gene Loss of the plasmid due to stochastic segregation in the case of DNA assembly technologies Homologous recombination, complex screening process to determine the clones that have correctly integrated the heterologous genotype into their sequence
Molecular genetics: metabolic engineering processes	The desired strain can be obtained in an almost "ad hoc" manner It can obtain hybrids more rapidly than other traditional methods Specific modifications of the genetic information and its corresponding phenotype	These techniques require an exhaustive knowledge of the metabolic pathways involved in the processes we want to alter
Random mutagenesis: mutation of yeast genes through UV light mutagenesis		Mutations randomly and complex and laborious process of selection The mutated hybrids may contain other undesired mutations that could result in technical or sensory inconvenience
Random mutagenesis: mutation of yeast genes through chemical mutagens		Mutations randomly and complex and laborious process of selection The mutated hybrids may contain other undesired mutations that could result in technical or sensory inconvenience

As we can see, in addition to the naturally occurring microorganisms that are used for beer production, the targeted genetic modification of these can give rise to microorganisms that incorporate or miss certain genes, so that they exert a specific influence on the synthesis of certain molecules responsible to provide beer with exclusive attributes. As we have already mentioned, one of the methods used to vary the genome of a yeast consists in the induction of mutagenesis by means of UV radiation with the aim of generating phenotypes of interest. However, this mutagenesis is not a directed method and in most cases, undesired genetic modifications and phenotypes are obtained [40].

Targeted genetic engineering: creating genetically modified organisms (GMO) yeasts

This is where genetic engineering comes in as a method to directly modify genetics through deletions, overexpressions or modulations. Targeted mutagenesis allows to enhance positive attributes, such as fermentation efficiency under high gravity conditions, increased production of aromatic compounds, improved ethanol production or decreased acetic acid production [40].

Nevertheless, genetic modification also has its drawbacks in terms of legislation, on the one hand, and also in terms of the need to modify structural and regulatory genes in addition to the coding gene to achieve the desired phenotype [44]. This is a fact of great importance and of extreme difficulty since, quite often, while targeting a very specific objective, a rather poor-quality product is obtained instead. Moreover, the genetic modification of industrial yeasts, which are usually polyploid, aneuploid or even alloploid, is a very complex task. This is mostly because laboratory-directed genetic modifications usually give good results when working on common haploid laboratory strains and not on industrial strains that have such an extensive and complex chromosomal composition [16]. In addition, the commercial acceptance of the product may be affected by the controversy associated to the use of GMOs (genetically modified organisms). Some studies suggest that the acceptance of GMO-produced products is low and has been declining in recent years [45]. Furthermore, beer is perceived as a natural product; hence, when this idea is somehow altered, consumers may react in a rather negative manner [46].

Regardless of the controversy around the use of GMOs for beer brewing, these organisms have continued to evolve, at least at laboratory level. Perhaps consumers' perception of these genetically modified strains could be more positive if the focus is placed on health benefits.

One of the instances where genetic engineering has been used consists in obtaining strains that degrade complex carbohydrates. Low carbohydrate content, i.e., light beers, without any shift in their aroma or flavor have been obtained. The process has been based on introducing genes from microorganisms such as *Saccharomyces cerevisiae diastaticus*, *Schwanniomyces occidentalis*, *Bacillus amyloliquefaciens* or *Aspergillus* among others [40] into the usual yeasts by means of recombinant DNA technology. There are problems related to this type of modifications, as we need a high degree of fermentation of the carbohydrates to reduce their final concentration in the final product. This more intense fermentation may lead to high alcohol contents in the final product. These drawbacks can be mitigated by modifying the wort maceration process [1]. The α-amylase gene has also been successfully introduced into yeast by means of this same technique and a 25% reduction in residual oligosaccharides has been achieved [37]. Genetic engineering has not only been used to reduce carbohydrate content, but also to lower ethanol production, and thus obtaining alcohol-free beers without the need to implement any additional dealcoholization process [47].

In the field of beer taste and flavor characteristics, some genetic modifications have also been carried out. An example of this is the increment of the β-lyase activity in yeasts through the use of CRISPR/CAS-9, the strains were able to produce beers with elevated levels of aromatic thiol compounds or "hop flavor". [48]. Diacetyl could be another example, since it is a compound that produces an undesirable buttery flavor, but when found at small concentrations, it plays an important role in the maturation of the beer and has, therefore, in those cases, a positive effect. The final concentration of diacetyl in beer depends on three factors: the synthesis and excretion of α-acetolactate, the enzymatic conversion of this precursor into diacetyl and the enzymatic removal of diacetyl [44]. One of the attempts to reduce this compound has been centered on introducing a gene from Enterobacter aerogenes into yeast by means of recombinant DNA techniques, so that it produces α-acetolactate decarboxylases (that decarboxylate and degrade α-acetolactate), while α-acetolactate (a diacetyl precursor) is eliminated and less diacetyl is produced [49]. Other alternatives have been found more recently, such as the design of a yeast that metabolizes α-acetolactate before expelling it from the cell or the use of yeasts with a damaged gene encoding acetolactate synthase, thereby decreasing the production of acetolactate [16]. More alternatives have been described that include the reduction of the activity of the enzyme *ILV2*, that generates α-acetolactate from pyruvate [44].

Sulfites are other compounds that play an important role in beer flavor. They capture carbonyls and form sulfite-carbonyl complexes that exhibit higher detection limits, in terms of flavor, than carbonyls on their own. By reducing

the relevance of carbonyls, they achieve the modification of food's flavor. In addition, they prevent the oxidation of beer once it has been bottled, thereby stabilizing its flavor and aroma [44]. Therefore, we have tried to increase sulfite concentrations, for example, by overexpressing the genes involved in the assimilatory reduction of sulfate into sulfide by the yeast, through which sulfite is produced. This overexpression has been accomplished by the inclusion in *S. cerevisiae* strains of plasmid constructs containing multiple copies of the *MET 14* and *MET 16* genes responsible for the formation of these sulfites [50, 51]. Hydrogen sulfide is one of the sulfur compounds that is undesirable in beer, as it adversely affects its flavor, even when it is present in small amounts. One of the options that has been studied to reduce its concentration in beer involves the overexpression of the *CYS4* gene, which encodes cystathionine β-synthase. This enzyme catalyzes the transformation of homocysteine into cystathionine when synthesizing cysteine, a reaction that requires hydrogen sulfide to be present. Thanks to the overexpression of this gene, a greater usage of hydrogen sulfide by the yeast is achieved, which in turn reduces the amount of this undesirable molecule in the final beer [52]. The taste of cooked vegetables produced by dimethyl sulfide (DMS) is another flavor that genetic engineering has tried to eliminate from beer. Dimethyl sulfide arises from the reduction of dimethyl sulfoxide (DMSO). Most research studies have focused on preventing such conversion, thus preventing the accumulation of dimethyl sulfide in the final product. The *MXR1* gene encodes the enzyme that catalyzes the reduction reaction of DMSO into DMS. Consequently, by disrupting the reading pattern of the gene, its silencing has been achieved and the production of large amounts of DMS has been prevented [53].

In the same way that artificial or intentional genetic modifications are applied to improve the sensory properties of beer, certain random mutations can also worsen them, which is the case of the respiration-deficient yeasts, also known as "petites". These yeasts present a mutation that produces a defect in their respiratory chain and are unable to grow when using non-fermentable carbon sources (such as glycerol or ethanol). This has a negative effect on the flavor when this microorganism is present in large concentrations [54].

Esters are also important components in terms of beer flavor and aroma. Isoamyl acetate, for example, is an ester that contributes with banana flavor. This compound may or may not be of interest, depending on the characteristics that are being sought in each particular beer. That is why different studies have achieved, on the one hand, an overexpression of the *ATF1* gene encoding the acetyltransferase (enzyme that catalyzes the formation of isoamyl acetate), which is very useful if we want to increase the final concentrations of this ester and its associated organoleptic characteristics. On the other hand, certain yeast strains that possess a defective variant of this gene and, therefore, have a lower production of isoamyl acetate have been designed through genetic engineering [16]. The list of esters, in addition to the one mentioned above, is extensive, and they contribute with different attributes to beer, among which are those volatile esters that provide fruity aromas. In addition to the *ATF1* gene, there are other genes such as *ATF2* or *lg-ATF1* that also encode the enzymes involved in the production of esters related to other flavors and aromas such as, for example, rose and honey. In the same way as in the case of *ATF1*, the genetic engineering manipulation of these genes allows to modulate the final concentration of esters in beers and, consequently, their aroma and flavor [44].

Another study on this type of aromatic compounds [55] focused on the modification of esterase genes (an enzyme that breaks ester bonds) to prevent the degradation of these esters and, consequently, the loss of fruity aromas. For this purpose, CRISPR-Cas9 technique was used to delete, by targeted homologous recombination, the genes *IAH1* and *TIP1*, which are the ones responsible for encoding the esterases in *Saccharomyces cerevisiae*, and thus producing lager beer. After applying this method, the esterase activity in the new mutants was compared against the "wild type" strains and it was found to be lower in the CRISPR-Cas9-modified organisms. Consequently, and as expected, the relative abundance of acetates and ethyl esters increased. Other authors, such as Dong et al. [56] also managed to increase acetate production, in this case by introducing an *Escherichia coli* plasmid into a polyploid industrial yeast strain "S6". This plasmid contained the *ATF1* gene (alcohol acetyl transferase) and a yeast promoter (*PGK1*) that induced the gene overexpression. The tests conducted demonstrated a significant increment of the alcohol acetyl transferase activity in the modified strains, which led to the appearance of significant concentrations of ethyl acetate in the final product. On the other hand, other studies have recently been conducted to increase the flocculation capacity of yeasts at low temperature through the disruption of the action exerted by the *RIM21* gene. No significant loss of its functional characteristics was observed [57].

In addition to the yeast strain, another significant contributor to the flavor of beer is hops, which provide bitterness and what is known as "hoppy flavor". In this case, genetic engineering also appears to be able to make the yeast produce this flavor without the need to directly add hops over the production process. Denby et al. [58] succeeded in doing so. They created drop-in brewer's yeast strains capable of biosynthesizing monoterpenes, by incorporation of plant secondary metabolism genes (basil and mint geraniol and

linalool synthase genes) into industrial brewer's yeast, that gave rise to hop flavor in finished beer.

As for aroma, other studies have used the genetic modifications of certain aspects in an attempt to improve it. Thus, we have found a study that sought to increase, through genetic engineering, the availability of cytosolic NADH as a means to improve the aromatic profile of beer. To alter NADH levels, the *FDH1* gene was overexpressed in the lager yeast *M14* by transforming native *M14* strains using the recombinant plasmid that contains this gene. This strain encodes a formate dehydrogenase dependent on recombinant NAD^+ that finally catalyzes the conversion of NAD^+ molecules into NADH, resulting in the strain *M-FDH1*. It was found that this new strain was able to accumulate more NADH in the cytosol without notable differences in its fermentation performance neither in the growth of this strain. This increased amount and availability of cytosolic NADH resulted in a reorganization of the metabolic pathways that involve NADH as well as an acceleration of the NADH-dependent reactions. Consequently, the formation and degradation of certain molecules was altered, which resulted in a notable reduction of certain substances responsible for unpleasant odors in beer, such as acetaldehyde, diacetyl or acetoin, and in turn in a better aromatic profile [59]. There is another study that supports this achievement. In this case, on the same *M14* strain, different recombinant strains that overexpressed a large number of enzymes were obtained whose final result, as in the previous case, was an increase in the amount of cytosolic NADH [60]. Again, the reductive activity in the cell was increased and certain pathways were activated, which led to a lower production and presence of unwanted substances. In this case, instead of aroma, flavor was evaluated and it was found that the higher availability of NADH leads to a lower production of flavor-altering substances and, therefore, to a more stable flavor over time [60]. Similarly, recent studies employed DNA recombination techniques to replace the *ADHII (alcohol dehydrogenase II)* gene in *Saccharomyces cerevisiae* by a cluster of genes including *ADH II* itself and other genes such as *gamma-glutamylcysteine synthetase*. As a result, the circulating values of acetaldehyde decreased by 22% with the subsequent organoleptic benefits for the final product [61].

Some of these applications to beer are summarized in Table 2.

Concluding remarks

Yeasts have been associated to millenary biotechnological processes (production of beers, wines or bread) almost since the very moment that humans became sedentary, so we could consider them as the oldest "domesticated" microorganisms. During this long period of time, their role has evolved from mere biotechnological tools that are necessary for fermentation processes, to subtle instruments where each strain imprints its mark on the final product. In the course of the last century, knowledge and scientific advances in the field of Molecular Biology have allowed us to exploit the most desired and valued particular characteristics of each strain. First, in a gross manner, by breeding hybrids that conferred new characteristics to the strains, then by directly extracting the genes responsible for those characteristics and introducing them into other organisms, hence, generating in an almost surgical manner, new strains with new attributes.

This bibliographic review has addressed how an array of genetic engineering techniques applied to beer brewing can determine the organoleptic characteristics of the final beers. The path for innovation that biotechnology opens up to the world of brewing is a road still to be traveled. While it is true that some of the beers that are already produced are based on new or transgenic microorganisms as well as on other biotechnological solutions, the number of microorganisms that can ferment and that can be found from the time the barley is harvested to the time a beer is bottled is immense. Research is progressively increasing, although most studies remain to be conducted at a laboratory or pilot scale.

However, we cannot ignore the fact that none of these advances will be of any use if we are not able to transfer this knowledge to the manufacturing sector, and this is where we may be facing the main "bottleneck" of the whole system. No matter how many biotechnological solutions we can develop, if they are not accepted by society and assumed as a form of progress, industry will simply not make use of them, or if they are currently doing so, they may stop doing so in the face of consumer rejection. Therefore, a parallel process of raising awareness of the benefits associated with biotechnological solutions is necessary, both for the food industry, in general, and for the brewing industry, in particular. It is logical, therefore, that the application of the biotechnological solutions described here at the industrial level is still scarce (despite the fact that some countries allow the use of genetically modified yeast strains in their regulations), and it will remain so until the new microorganisms involved and the benefits that can be obtained from them are well known and accepted by society. Once this happens, the next task will be to put them to work at an industrial scale in the same routine way that *Saccharomyces sp* have been used for centuries.

Table 2 Some genetic modifications performed in microorganisms for the production of beers

Microorganism	Genetic modification	Improvement	References
Saccharomyces cerevisiae	Suppressing of gene *NHS5* (cystathionine β-synthase), responsible for the formation of hydrogen sulfide	Reduction of the formation of H_2S	Tezuka et al., 1992
Saccharomyces cerevisiae	Overexpression of *MET14* gene (APS kinase), responsible for the formation of sulphite, and the *SSU1* gene (high-affinity sulphate transport protein), responsible for transporting the sulphite created outside the cell	Increase in the production of sulphite	Donalies and Stahl, 2002
Saccharomyces cerevisiae	Deletion of genes *IAH1* and *TIP1* (esterases)	Increase in the production of esters, responsible for fruity aromas	Dank et al., 2018
Saccharomyces cerevisiae	Expression of *LIS* genes (linalool synthase) and *GES* genes (geraniol synthase). Alterations to endogenous yeast genes	Increase in the production of monoterpenes such as linalool and geraniol	Denby et al., 2018
Saccharomyces cerevisiae	Hybridization with *Saccharomyces arboricola, Saccharomyces eubayanus, Saccharomyces mikatae and Saccharomyces uvarum*	Better development of fermentation and higher production of alcohols and esters	Nikulin et al., 2018
Saccharomyces cerevisiae S6	Overexpression of *ATFI* gene (alcohol acetyl transferase)	Increase in the production of ethyl acetate	Dong et al., 2019
Saccharomyces cerevisiae ssp. *carlsbergensis*	Overexpression of *GPD1* gene (Glycerol-3-phosphate dehydrogenase)	Ethanol reduction in beer, without affecting the consumption of wort sugars	Nevoigt et al., 2002
Saccharomyces carlsbergensis IF00751	Introduction *ALDC* gene (α-acetolactate decarboxylase), responsible of conversion of α-acetolactate into acetoin	Reduction of the conversion of α-acetolactate into diacetyl, responsible foroff-flavor in beer	Fujii et al., 1990
Saccharomyces carlsbergensis	Deletion of *MXR1* gene (peptide methionine sulfoxide reductase)	Elimination of the production of dimethyl sulphide from dimethyl sulfoxide	Hansen et al., 2002
Saccharomyces pastorianus	Disruption of *ILV2* gene (α-acetolactate synthase) and introduction of *AMY* gene (α-amylase)	Reduction of α-acetolactate synthase activity, reducing the time needed to stop beer maturation, and to increase the capacity of using dextrins o starch as a carbon source	Liu et al., 2004
Saccharomyces sp.	Introduction of amylolytic enzymes genes	Ability of using dextrins or starch as a carbon source, increasing the capacity of producing ethanol, and maintaining beer flavor	Kyselová and Brányik, 2015
Brewing yeast *Saccharomyces* sp. strain *M14*	Overexpression of *FDH1* genes (NAD^+- dependent formate dehydrogenase)	Reduction of levels of compounds responsible for off-flavors such as acetaldehyde, diacetyl, and acetoin, improving beer aroma	Xu et al., 2019
Brewing yeast *Saccharomyces* sp. strain *M14*	Overexpression of *FDH1* gene (NAD^+- dependent formate dehydrogenase) or overexpressing *CIT1* and *CIT2* (citrate synthase) or overexpressing *IDH1* (isocitrate dehydrogenase)	Reduction of acetaldehyde levels	Xu et al., 2020

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Declarations

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