



Review

# Saccharomyces cerevisiae in the Production of Fermented Beverages

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**Abstract:** Alcoholic beverages are produced following the fermentation of sugars by yeasts, mainly (but not exclusively) strains of the species, *Saccharomyces cerevisiae*. The sugary starting materials may emanate from cereal starches (which require enzymatic pre-hydrolysis) in the case of beers and whiskies, sucrose-rich plants (molasses or sugar juice from sugarcane) in the case of rums, or from fruits (which do not require pre-hydrolysis) in the case of wines and brandies. In the presence of sugars, together with other essential nutrients such as amino acids, minerals and vitamins, *S. cerevisiae* will conduct fermentative metabolism to ethanol and carbon dioxide (as the primary fermentation metabolites) as the cells strive to make energy and regenerate the coenzyme NAD<sup>+</sup> under anaerobic conditions. Yeasts will also produce numerous secondary metabolites which act as important beverage flavour congeners, including higher alcohols, esters, carbonyls and sulphur compounds. These are very important in dictating the final flavour and aroma characteristics of beverages such as beer and wine, but also in distilled beverages such as whisky, rum and brandy. Therefore, yeasts are of vital importance in providing the alcohol content and the sensory profiles of such beverages. This Introductory Chapter reviews, in general, the growth, physiology and metabolism of *S. cerevisiae* in alcoholic beverage fermentations.

Keywords: Saccharomyces cerevisiae; fermented beverages

#### 1. Introduction

Yeasts in Alcoholic Beverage Fermentations

The production of alcoholic beverages from fermentable carbon sources by yeast is the oldest and most economically important of all biotechnologies. Yeast plays a vital role in the production of all alcoholic beverages (see Figure 1) and the selection of suitable yeast strains is essential not only to maximise alcohol yield, but also to maintain beverage sensory quality.

The yeast species that dominates in the production of alcoholic beverages worldwide is *Saccharomyces cerevisiae*, and the particular strains of this species employed in fermentation exert a profound influence on the flavour and aroma characteristics of different beverages. For large-scale beverage fermentations, as in brewing, winemaking and distilled spirit production, pure cultures of selected strains of *S. cerevisiae* are usually used. These strains are either sourced *in house* or supplied from yeast producing companies. In smaller-scale (artisanal) processes, spontaneous fermentations may be allowed to occur that rely on indigenous microbiological flora (wild yeasts and bacteria) present in the raw material and in the production facility. For example, this would be typical in small distilleries in Mexico (for Tequila and Mezcal production) and in Brazil (for Cachaça production). In some types of alcoholic beverage fermentations, non-*S. cerevisiae* yeasts may be employed either as starter cultures, or occur naturally. For example, in winemaking the *S. cerevisiae* yeast strain used

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to commence fermentation may be overrun by the indigenous yeast flora associated with the grapes. Table 1 summarises different yeast species encountered in alcoholic beverage fermentations.

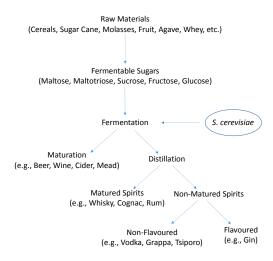


Figure 1. The key roles of Saccharomyces cerevisiae in production of fermented beverages.

**Table 1.** The main types of yeast used in the production of selected alcoholic beverages.

Beverage	Yeast Involved	Comments
Beer	Lager beer: Saccharomyces pastorianus Ale: Saccharomyces cerevisiae Lambic beer: Brettanomyces bruxellensis and other yeasts	Lager yeasts are likely a natural hybrid ( <i>S. cerevisiae &amp; S. eubayanus</i> ). Relatively few strains employed in lager fermentations. Lager strains utilise maltotriose more efficiently than ale strains, and they ferment at cooler temperatures. Ale yeasts are polyploid strains. Numerous strains employed in ale brewing. Ale yeasts ferment at warmer temperatures compared with lager yeasts. Lambic beer traditionally produced via spontaneous fermentation, but some lambic and Belgian-style beers employ pure starter cultures of <i>Brettanomyces</i> spp.
Wine	Saccharomyces cerevisiae, Saccharomyces bayanus (pure cultures) and naturally-occurring yeasts	Traditional winemaking is characterised by spontaneous fermentations of grape must with naturally occurring microflora (the main yeast genera associated with grapes are: Kloeckera and Hanseniaspora, with lesser representations of Candida, Metchnikowia, Cryptococcus, Pichia and Kluyveromyces and very low populations of Saccharomyces cerevisiae) Modern, large-scale wineries use specially selected starter cultures of S. cerevisiae strains available in dried form (e.g., active dry yeast, ADY) from specialist yeast supply companies. Occasionally, secondary commercial non-Saccharomyces starter cultures (e.g., Candida stellata) may be employed to impart specific flavour and aroma to wine.
Whisky	Saccharomyces cerevisiae	Scotch whisky producers currently use selected distilling strains of <i>S. cerevisiae</i> in three main formats, cream yeast, pressed (cake) and dried yeast. Malt whisky distilleries traditionally use pressed yeast, but larger grain distillers have now adopted cream yeast. Dried yeasts are not as prevalent as pressed and cream formats in whisky fermentations.
Rum	Saccharomyces cerevisiae and Schizosaccharomyces pombe	S. cerevisiae strains in rum fermentations are developed as starter cultures and provide faster fermentation with more higher alcohols and fatty acids, but less esters resulting in lighter style rums.  Schiz. pombe in rum fermentations provides slower fermentations leading to less higher alcohols and fatty acids, but more esters resulting in heavy, strong aroma rums. Growth of Schiz. pombe is favoured by low pH, higher sugar conc.
Tequila, Mezcal, Bacanora	Natural yeasts in artisanal Agave fermentations	Various yeasts have been isolated from such processes: <i>S. cerevisiae</i> , <i>Kluyveromyces marxianus</i> , <i>Pichia</i> spp., <i>Brettanomyces</i> spp., <i>Rhodotorula</i> spp., etc.
Brandies, Gin, Vodka, etc.	Saccharomyces cerevisiae	For brandies, cognac, etc. the base wine is produced by pure starter cultures of <i>S. cerevisiae</i> . For gin, vodka, etc. selected distilling strains of <i>S. cerevisiae</i> will be used.
Cheese whey-derived beverages	Kluyveromyces marxianus	Lactose-fermenting yeast to produce ethanol destined for gin, vodka and cream liqueurs, etc.

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Brewing yeasts are members of the *Saccharomyces* genus, but two different species are utilised for ale and lager brewing. Regarding the former, these are strains of *S. cerevisiae* that traditionally conduct "top fermentations" where yeasts congregate on the surface of the fermenting wort. Some non-flocculent ale yeasts may be employed if centrifuges are used in yeast harvesting. There are numerous different strains of ale yeasts which have complex genetic (aneuploid or polyploid) characteristics. Lager yeast strains are hybrids of the species *S. cerevisiae* and *S. eubayanus* and are now designated as *Saccharomyces pastorianus*. These yeast cultures conduct "bottom fermentations" and usually have a tendency for flocculation. This facilitates cropping of yeast at the end of fermentation on the bottom of a fermenter usually in the cones of cylindro-conical vessels. The roles of yeast in beer production are covered by Stewart in the following article of this Special Issue.

Winemaking involves the extraction of grape juice ('must') followed by yeast (natural yeasts or commercial starter cultures of *S. cerevisiae*) fermentation. Bacterial malolactic fermentation may also take place (where malic acid is converted to lactic acid), followed by aging, clarification and packaging. Wine yeast starter cultures are predominantly strains of *S. cerevisiae*, but some commercial non-*Saccharomyces* starter cultures (for example, *Candida stellata*) may be employed to impart specific flavour and aroma characteristics to wine [1]. The role of *S. cerevisiae* in wine production is considered by Pretorius in this Special Issue.

Fermentations for the production of whisky and other distilled spirits derived from cereals are conducted by specific strains of *Saccharomyces cerevisiae* which convert mash sugars into ethanol, carbon dioxide and numerous secondary fermentation metabolites that collectively act as flavour congeners in the final spirit [2]. Therefore, the choice of yeast strain that can contribute significantly to the organoleptic qualities of spirits is critical! The extracted sugars following cereal mashing are predominantly maltose and maltotriose, in contrast to glucose, fructose and sucrose in wine must that are liberated from grape crushing. In malt and grain Scotch whisky production, where no exogenous enzymes are allowed according to the Scotch Whisky Regulations of 2009, small branched maltodextrin molecules remain in the wort following mashing and some whisky yeast strains are available that may further utilise these oligosaccharides. However, unlike brewing, the wort is not boiled and therefore will contain amylolytic activity from malt. A widely used Scotch whisky yeast strain, named "M type"—thought to be a hybrid of *S. cerevisiae* and *S. diastaticus*—possesses limited amylolytic activity. The role of *S. cerevisiae* in the production of whisky is covered in greater detail by Walker and Hill in this Special Issue.

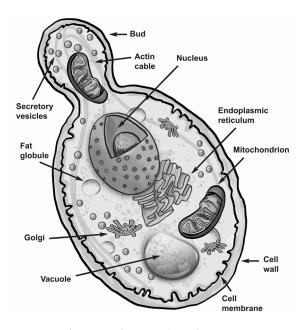


Figure 2. Main features of a typical Saccharomyces cerevisiae cell.

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### 2. Physiology of Alcohol-Producing S. cerevisiae

### 2.1. Form and Function of S. cerevisiae Cells

 $S.\ cerevisiae$ , the predominant yeast employed in fermented beverage production, is generally ellipsoid in shape with a large diameter of 5–10  $\mu m$  and a smaller diameter of around 5  $\mu m$ . All yeasts are unicellular fungi that have ultrastructure features similar to that of higher eukaryotic cells. That is, they comprise a cell wall, nucleus, mitochondria, endoplasmic reticulum (ER), Golgi apparatus, vacuoles, microbodies, and secretory vesicles together with a complex extracellular and intracellular membrane network (Figure 2).  $S.\ cerevisiae$  cytology has been discussed in detail by Walker [3,4].

# 2.2. Physical Requirements of S. cerevisiae

High water activity is required for S. cerevisiae cells which typically possess a minimum  $a_w$  of around 0.65. Water is absolutely essential for fermentation, and high sugar-containing media can impose osmotic stress (reduced water availability) on cells to adversely affect cell physiology. This is frequently encountered in brewing in so-called very high gravity (VHG) fermentations and increasingly in whisky production. Yeast cells can respond to the lack of water by overproducing glycerol, or other osmolytes such as trehalose which act to protect yeast membranes from desiccation. Such compounds can effectively replace cellular water, restore cell volume and enable yeast metabolism to continue. However, over-production of glycerol can (albeit not always) detract from ethanol yield in high gravity fermentations.

Regarding temperature and pH requirements for alcoholic fermentations, yeasts thrive in warm and acidic environments with most *S. cerevisiae* strains growing well between 20 and 30 °C and pH 4.5 and 6.5. Exceptions to these characteristics are lager brewing yeast strains of the species *S. pastorianus* which are adapted to ferment in cooler temperatures (for example, 8–15 °C). Fermenting yeasts acidify their growth environment through a combination of proton secretion during nutrient transport (through the action of the plasma membrane proton-pumping ATPase), direct secretion of organic acids (for example, succinate and acetate), removal of buffering agents and carbon dioxide evolution and dissolution.

Regarding oxygen requirements, although *S. cerevisiae* is sometimes referred to as a facultative anaerobe, this yeast cannot actually grow under strictly anaerobic conditions. This is because oxygen is absolutely required as a growth factor for membrane fatty acid (for example, oleic acid) and sterol (for example, ergosterol) biosynthesis. *S. cerevisiae* is auxotrophic for oleic acid and ergosterol under anaerobic conditions. Therefore, for effective alcoholic fermentations, either some oxygen can be supplied at the start of fermentation, or fatty acids and sterol growth factors can be supplemented to the medium (using commercially available yeast foods).

#### 2.3. Nutritional Requirements of S. cerevisiae

For the production of alcoholic beverages by *S. cerevisiae*, the nutrient composition of the fermentation medium is crucially important for yeast growth and metabolism and, concomitantly, the quality of the final beverage. The cost of the medium is also important – raw materials account for a significant proportion (generally over 50%) of the overall costs of fermented beverage production. It should be noted that it is not solely sugars that impact on yeast fermentation performance. Yeasts also require appropriate supplies of other major, minor and trace nutrients, together with water, in order to efficiently carry out fermentation. Most *S. cerevisiae* strains can grow if supplied with glucose, ammonium salts, inorganic ions and a few growth factors. Macronutrients need to be supplied at millimolar concentrations, and these comprise sources of carbon (i.e., sugars), free amino nitrogen (amino acids, small peptides and ammonium salts), oxygen, sulphur, phosphorus, potassium and magnesium. Micronutrients are only needed by yeast at micromolar concentrations, and they comprise trace elements such as calcium, copper, iron, manganese and zinc. Sources of nutrients in the media, commonly employed in the production of fermented beverages, are described below.

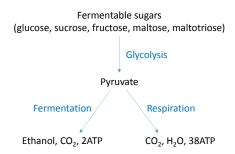
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#### 3. Yeast Nutrition

# 3.1. Nutrients Required by S. cerevisiae

#### 3.1.1. Sources of Utilizable Carbon

Yeasts are chemoorganotrophic microfungi that obtain their carbon and energy by metabolizing organic substrates. Although glucose is commonly used as the sole carbon source for yeast growth in the laboratory, this sugar is not generally freely available in industrial fermentation media. In such media, reviewed by Walker (2014) [5], the more common carbon sources are maltose (as in malt wort for brewing), sucrose (as in molasses for rum production), lactose (as in cheese whey-based beverages) and fructose (as in *Agave* spp. polyfructans for Tequila). Only in fruit juices and wine must will free glucose be available, together with fructose [5]. The metabolic fate of sugars in yeast fermentative metabolism is outlined in Figure 3.



**Figure 3.** The fate of sugars during *S. cerevisiae* metabolism.

## 3.1.2. Sources of Utilizable Nitrogen

*S. cerevisiae* cannot fix atmospheric nitrogen (being non-diazotrophic), and require a supply of readily assimilable organic nitrogen (for example, amino acids) or inorganic nitrogen (for example, ammonium salts) for growth and fermentative metabolism. Urea can also be utilised by yeast, but this nitrogen source should not be a supplement for beverage fermentation media owing to the possible formation of carcinogenic ethyl carbamate. Nitrogen in yeast fermentation media serves an anabolic role for the biosynthesis of structural and functional proteins (enzymes) and nucleic acids, and a catabolic role in the production of fermentation flavour congeners such as higher alcohols. For distillery yeasts, levels of free alpha-amino nitrogen (FAN) can be growth limiting and Ingledew [6] has reported that the growth of distilling strains of *S. cerevisiae* increases almost linearly with FAN levels up to 100 mg/L.

# 3.1.3. Sources of Inorganic Nutrients

In addition to the sugar and nitrogen sources in fermentation media, yeasts also need the correct supply of inorganic ions. Minerals, especially key metal ions, are often overlooked as important determinants of yeast fermentation performance, in spite of the fact that the nature and concentration of metal ions supplied in growth media can have a significant impact on yeast fermentations. Phosphorus, sulphur, potassium and magnesium are key examples of "bulk" minerals required in millimolar concentrations, whilst sodium, calcium, iron, cobalt, zinc, molydenum, copper, manganese, nickel and selenium are "trace" elements required in micromolar, or less, concentrations [7].

Complex fermentation media used for fermented beverage production (for example, malt wort, molasses, wine must, cheese whey) normally contains adequate levels of inorganic ions for yeast growth, but supplementation with additional minerals may occasionally be necessary (for example, zinc may be deficient). Also, the *bioavailability* of metal ions in complex industrial fermentation media may be compromised due to precipitation, chelation or absorption. Regarding zinc, in alcoholic fermentations, this metal is particularly important as it is an essential cofactor of the terminal

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alcohologenic enzyme, ethanol (alcohol) dehydrogenase. Consequently, media deficient in zinc may result in sluggish or stuck fermentations, and this has long been recognised as an occasional problem in the brewing industry. Regarding magnesium, this is absolutely essential for yeast in the production of ethanol and it is important to maintain high levels of bioavailable magnesium to ensure maximal fermentation performance [7,8].

Regarding other important inorganic nutrient requirements, yeasts can synthesize sulphur amino acids from sulphate, the most oxidized form of inorganic sulphur. Phosphorus can be provided to yeasts in the form of phosphate salts and this is essential for the biosynthesis of nucleic acids, phospholipids and ATP. The phosphate content of yeast cells is approximately 3%-5% of dry weight and this is mainly in the form of orthophosphate ( $H_2PO_4^-$ ) which acts as a substrate and enzyme effector. The yeast vacuole (depicted on Figure 2) can serve as a storage site for phosphate in the form of polyphosphates (or volutin granules).

#### 3.1.4. Sources of Growth Factors

S. cerevisiae requires growth factors at very low concentrations in order to perform specific catalytic or structural roles and these include vitamins, purines and pyrimidines, nucleotides and nucleosides, amino acids, fatty acids and sterols. Complex media, such as malt wort or wine must, should be able to provide these accessory growth factors for alcohol fermentations, but commercially available yeast "foods" may also be employed to supplement media. These are based on mixtures of yeast extract, ammonium phosphate and minerals (for example, magnesium and zinc) and may be employed in alcohol fermentations to ensure consistent yeast activity. Blackstrap molasses for rum production may occasionally be deficient in pantothenic acid and inositol.

## 3.2. Nutrient Composition of Fermentation Media

Sources of sugars for beverage fermentations can either be directly extracted from sugar-rich plants (for example, from sugarcane in the case of molasses or fruits in the case of wine must) or from starch-rich plants (for example, following pre-hydrolysis of cereal starches from barley, maize, and wheat). During hydrolysis of starch with malt amylase enzymes, higher saccharides such as oligosaccharides (for example, maltodextrins) are liberated that are typically not utilised by *Saccharomyces* yeasts employed for fermented beverage production.

Table 2 lists the main types of fermentation media employed in the production of alcoholic beverages.

Media	Fermentable Sugars	Beverage	
Barley malt wort	Glucose, maltose, maltotriose	Ale and lager beer. Scotch malt whisky	
Cereal wort based on barley malt and exogenous enzymes plus un-malted starch from wheat, rye, maize, sorghum, etc.	Glucose, maltose, maltotriose	Some beers, Scotch grain whisky, Bourbon and Tennessee whiskey, Canadian rye whisky, Irish whiskey, grain neutral spirit (for gin, vodka, etc.)	
Rice hydrolysate (from Koji enzymes)	Glucose	Sake, Sochu, Arrack, Awamori	
Potato hydrolysate (from amylolytic enzymes)	Glucose	Aquavit, vodka	
Agave	Fructose	Tequila, mezcal, pulque	
Sugar cane molasses	Sucrose Rum		
Sugar cane juice	Sucrose	Cachaça (Brazil), Rhum Agricole	
Grape must, fruit juices, honey	Glucose, fructose	Wine, cognac, armagnac, brandy, grappa, kirsch, slivovich, cider, perry, mead	
Cheese whey	Lactose	Gin, vodka, cream liquers	

Table 2. Some fermentation media for alcoholic beverages.

More detailed information on the composition of selected fermentation media for alcoholic beverage production is provided in Table 3 and the topic has been extensively reviewed [5].

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Component	Molasses	Malt Wort	Grape Must	Cheese Whey
Carbon source	Sucrose, glucose, fructose, raffinose	Glucose, maltose, maltotriose	Glucose, fructose	Lactose
Nitrogen source	Limiting (requires supplementation)	Amino acids	Amino acids	Amino and urea nitrogen, albumins, globulins
Minerals	P, S adequate, but K and Ca may be in excess. Mg and Mn may be limiting	Most minerals adequate, but Zn may be limiting	Most minerals adequate, but Zn, Mg may be limiting	Most minerals adequate
Vitamins	Most vitamins adequate, but biotin may be limiting	Most vitamins adequate, but biotin may be limiting	Range of vitamins present but yeast foods may supply extra	Biotin, pyridoxine and thiamine present
Minor components	Maillard reaction products, betaine, organic acids, waxes, pigments, silica	Maltodextrins (unfermentable)	Pentoses (unfermentable), organic and fatty acids	High levels of lactic acid, fat, and fibre

Table 3. Main components of selected fermentation media for production of alcoholic beverages.

#### 3.3. Nutrient Uptake by S. cerevisiae

The yeast plasma membrane is the cellular barrier which dictates nutrient entry into cells and which plays an important role in governing the rates of yeast growth and fermentation. Nutrients are transported into yeast cells across the plasma membrane via mechanisms such as simple net diffusion (a passive or free mechanism), facilitated (catalysed) diffusion, diffusion channels and active (energy-dependent) transport. This latter mechanism involves the activity of plasma membrane ATPases that act as directional proton pumps creating pH gradients that drive nutrient transport either via proton symporters (as is the case with certain sugars and amino acids) or via proton antiporters (as is the case with potassium ions).

For sugars, the precise mode of translocation by *S. cerevisiae* will depend on the sugar being used, yeast species, and fermentation conditions. For example, glucose is transported by facilitated diffusion and maltose by active transport. In fermentation media such as malt wort, glucose exhibits a repressive effect on the assimilation of other sugars (such as maltose) by *S. cerevisiae*, a phenomenon known as catabolite repression. This may result in slow or incomplete fermentations and the production of off-flavours in beverages [9]. For example, in brewer's wort, Berry and Slaughter [10,11] have described a "maltose lag" due to glucose repression of maltose uptake which may result in "stuck fermentations" if malt wort is supplemented with glucose adjuncts.

Regarding amino acid uptake by yeast, they represent the preferred nitrogen sources for *S. cerevisiae* in fermentation media and are assimilated sequentially by yeast cells, but the presence of ammonium ions may inhibit their uptake due to nitrogen catabolite repression. In *S. cerevisiae*, two classes of energy-dependent amino acid uptake systems operate: one is broadly specific (the general amino acid permease, GAP) and effects the uptake of all naturally occurring amino acids, whilst the other includes a variety of transporters that display specificity for particular amino acids. *S. cerevisiae* can also dissimilate amino acids (by decarboxylation, transamination, or fermentation) to yield ammonium, glutamate, and higher alcohols (fusel oils).

# 3.4. S. cerevisiae Growth During Fermentation

Yeast growth involves transport and assimilation of nutrients followed by their integration into numerous cellular components in order for cells to increase in biomass and eventually divide. The primary aim of a yeast cell is to reproduce, rather than to make alcohol. Nevertheless, during beverage fermentations, alcohol production and yeast growth are inextricably linked. Ethanol is produced as cells strive to maintain their redox balance and make sufficient ATP for continued growth. In fact, ethanol cannot be produced efficiently without significant growth of yeast cells.

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Non-growing yeast cells will ferment only sufficient sugar to produce energy for cell maintenance. Consequently, the dilemma facing distillers, brewers and winemakers is to provide sufficient nutrients for yeast cultures in order to carry out fermentation whilst, at the same time, avoiding excessive yeast growth which will represent alcohol loss. Compromise efforts can be adopted to minimise yeast growth during alcoholic fermentation by employing: high yeast cell densities/cell re-cycle systems, continuous/semi-continuous fermentations, and/or immobilised yeast bioreactors.

*S. cerevisiae* reproduces vegetatively by multi-lateral budding in which daughter buds emanate from different locations on the mother cell surface. Yeast buds are initiated when mother cells attain a critical cell size at a time that coincides with the onset of DNA synthesis. When dividing yeast cells separate, scar tissue rich (Figure 2) in chitin (a polymer of N-acetyl glucosamine) is left on the yeast cell surface. These are known as the bud and birth scars, and they remain on the daughter bud and mother cells, respectively.

When *S. cerevisiae* cells are pitched into fresh fermentation media and incubated under optimal physical growth conditions, a typical batch growth curve will result which comprises the lag phase (period of no growth, but physiological adaptation of cells to their new environment), an exponential phase (limited period of logarithmic cell doublings), and a stationary phase (resting period with zero growth rate). During fermentation, the period of maximum sugar uptake and alcohol production coincides with the logarithmic phase, and it has been calculated that growing yeast cells produce ethanol 33 times faster than non-growing cells [6].

#### 4. S. cerevisiae Fermentative Metabolism

# 4.1. Ethanol Fermentation by S. cerevisiae

Fermentative yeasts are able to use sugars anaerobically as electron donors, electron acceptors, and carbon sources. *S. cerevisiae* is regarded as an ethanologenic yeast that can readily ferment glucose, fructose, mannose, galactose, sucrose, maltose and maltotriose into ethanol and carbon dioxide. The sequence of enzyme-catalyzed reactions that convert glucose to pyruvic acid is known as glycolysis and can be summarised as:

Glucose + 2ADP + 2Pi + 2NAD
$$^+$$
  $\rightarrow$  2Pyruvate + 2ATP + 2NADH $^+$  + 2H $^+$ 

This pathway provides yeast cells with energy and reducing power (in the form of NADH) for growth. In glycolysis, glucose is firstly phosphorylated using ATP to produce fructose 1,6-biphosphate which is then split by aldolase to form two triose phosphate compounds. Additional phosphorylation forms two triose diphosphates from which four H atoms are accepted by two molecules of NAD<sup>+</sup>. In the latter stages of glycolysis, four molecules of ATP are formed and this results in the formation of two molecules of pyruvic acid, with 2 molecules net of ATP produced. This is the only source of energy obtained by *S. cerevisiae* during fermentative metabolism. Fermentation is thus less energetically favourable compared with respiratory metabolism which, in *S. cerevisiae*, will only occur in aerobic conditions when glucose levels are kept very low (due to the Crabtree effect) [3]. A good example of the glycolytic pathway in brewer's yeast is provided in the following chapter.

In yeast cells undergoing alcoholic fermentation of sugars under anaerobic conditions, NAD<sup>+</sup> is regenerated in terminal step reactions from pyruvate. This regeneration of NAD<sup>+</sup> is necessary to maintain the redox balance and to enable glycolysis to continue. In the first of these, pyruvate is decarboxylated (by pyruvate decarboxylase) before a final reduction, catalyzed by alcohol dehydrogenase (ADH) to ethanol:

2Pyruvate + 2NADH + 
$$2H^+ \rightarrow 2NAD^+ + 2Ethanol + 2CO_2$$

The intermediate compound, that is formed in this reaction, acetaldehyde, acts as the electron acceptor and is generated following pyruvate decarboxylation:

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$$\begin{array}{ccccc} CH_3COCOOH & \stackrel{Pyruvate\ decarboxylase}{\rightarrow} & CH_3CHO + CO_2 & \stackrel{Alcohol\ dehydrogenase}{\rightarrow} & CH_3CH_2OH \\ Pyruvate & Acetaldehyde + CO_2 & Ethanol \\ \end{array}$$

NAD<sup>+</sup> is regenerated by alcohol (ethanol) dehydrogenase which requires zinc as an essential co-factor for its activity. As already discussed, if zinc is limiting in the fermentation media, the rate and extent of alcohol production may be compromised due to this important co-factor requirement.

The theoretical (stoichiometric) conversion to ethanol from glucose is as follows:

$$\begin{array}{cccc} C_6H_{12}O_6 & \rightarrow & 2C_2H_5OH & + 2CO_2 \\ & & & & & & & \\ I80 \text{ kg} & & 92 \text{ kg} & & 88 \text{ kg} \end{array}$$

Therefore, for each kilogram of glucose fermented, approximately 500 g of ethanol can theoretically be produced. However, in industrial fermentations, the best yields are only ~90% of this theoretical conversion equation due to some fermentable carbon being diverted to new yeast biomass and for the biosynthesis of minor fermentation metabolites (flavouring compounds, etc.).

# 4.2. Production of Secondary Fermentation Metabolites

Secondary fermentation metabolites of *S. cerevisiae* include: higher alcohols, polyols, esters, organic acids, vicinal diketones, and aldehydes [12] (see Table 4). These metabolites, although they are produced by yeast in much lower concentrations compared with ethanol and carbon dioxide, are very important flavour congeners in fermented beverages.

<b>Table 4.</b> Yeast seconda	ary fermentation metal	abolites as beverage congeners	•

Metabolite Class	Examples of Compounds	Comments
Higher alcohols	Isoamyl alcohol, Phenylethanol, Isopropanol	Within certain concentration limits, higher alcohols (or fusel oils) impart desirable flavour and aromas to fermented beverages, notably in distilled spirits.
Esters	Ethyl acetate	These compound impart fruity and floral flavours and aromas to fermented beverages, especially beers and wines.
Carbonyl compounds	Acetaldehyde	Above its flavour threshold in beer, this compound can impart a "grassy" or "green apple" flavour but this can be removed by secondary yeast fermentation during conditioning.
Organic acids	Succinic acid, Citric acid, Acetic acid	These compounds contribute in a beneficial way to the astringency, or "sharpness", of fermented beverages. The presence of some acids, notably lactic acid, indicate undesirable bacterial spoilage.
Polyols	Glycerol	This compound is produced during normal yeast metabolism, or when yeasts are confronted with osmotic stress. Glycerol may contribute desirable viscosity to fermented beverages, notably wines.
Vicinal diketones	Diacetyl, Pentane-2,3-dione	Diacetyl in most beers is undesirable, imparting a rancid-butter or "butterscotch" flavour, but levels can be reduced during beer conditioning.
Sulphur compounds	Hydrogen sulphide, Dimethyl sulphide, Sulphur dioxide, Thiols	These are important beverage flavour and aroma compounds. For example in beer, dimethyl sulphide (DMS) if present in low concentrations is a desirable attribute of lagers, but higher concentrations impart off-flavours.
Phenolic compounds	4-Vinylguiacol	Some yeasts, including wild yeasts, that are <i>POF</i> + (phenolic off-flavour) can produce undesirable phenolic flavours and aromas. However, the clove-like compound, 4-vinylguiacol, is desirable in certain beer styles and can be produced by hefe ale yeast strains of <i>S. cerevisiae</i> .

Two of the main secondary fermentation metabolites are glycerol and succinic acid. Some winemakers consider the presence of glycerol in wine to impart "mouthfeel", as a contributor to the viscosity of the beverage [1]. After ethanol and CO<sub>2</sub>, glycerol is the largest concentration metabolite. Succinic acid may be produced by *S. cerevisiae* following limited operation of the citric acid cycle

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(TCA cycle), whereas glycerol may be produced in redox-balancing reactions, or as a compatible solute in response to osmotic stress [13]. Regarding esters, these compounds represent a very important group of flavour-active compounds that generally result in desirable fruity/floral flavours and aromas in fermented beverages. Most of these are produced by yeast during fermentation in reactions between alcohols and acyl CoA molecules but some esters, notably ethyl lactate, are linked to bacterial spoilage (for example, from *Lactobacillus* spp.). The concentration of esters produced during fermentation depends on the relative abundance of the corresponding alcohols and acyl CoAs, but since acetyl CoA and ethanol are the most abundant, ethyl acetate is the predominant ester produced. Isoamyl acetate is produced in lower concentrations, but as it has a much lower flavour threshold than ethyl acetate, it is a more significant contributor to beverage aroma.

Diacetyl is a vicinal diketone produced by *S. cerevisiae* during a side reaction in the synthesis of the amino acid valine. It may also be present in beer due to spoilage by contaminant lactic acid bacteria. In brewing, this low flavour threshold compound (the taste threshold of diacetyl in lager beer is around 0.1 mg/L) is reduced by yeast towards the end of fermentation and during beer conditioning. The presence of diacetyl, particularly in lager-style beer, is undesirable as it imparts a rancid-butter flavour sometimes referred to being akin to butterscotch.

The production of higher alcohols or *fusel oils* during fermentation follows amino acid deamination and decarboxylation, known as the Ehrlich Pathway, a catabolic route which comes into play when there are excess amino acids in the medium. When these are deficient, an alternative anabolic route called the Biosynthesis Pathway becomes operational deriving higher alcohols from  $\alpha$ -keto acid intermediates [3,14]. Higher fermentation temperatures tend to favour increased levels of fusel oils. Fusel oils are especially important in flavour development in alcoholic beverages such as beer, wine and whisky, and the levels of individual fusel oils in beverages are linked to the levels of corresponding amino acids in the fermentation medium (for example, phenylalanine stimulates phenylethanol production leading to a rose-like aroma). In distilled beverages, these compounds, within certain concentration limits, make a particularly important beneficial contribution to the aroma characteristics of distillates.

Certain yeasts, generically termed *wild yeasts*, can also affect the organoleptic qualities of fermented beverages as contaminant microorganisms during fermentation and in finished products. These include wild strains of *S. cerevisiae*, other members of the *Saccharomyces* genus, and numerous other non-*Saccharomyces* yeasts and have been reviewed by Stratford [15] and Deak [16]. Regarding bacteria, generally, their presence during yeast alcoholic fermentations is regarded as being detrimental with regard to both detracting from ethanol yields, and compromising product quality especially in beer (unacceptable flavour, poor foam stability and unsatisfactory physical stability). The predominant contaminating bacteria are lactic acid bacteria, but occasional problems can also arise from acetic acid bacteria and wild yeast contamination. These microorganisms can cause a variety of flavour (organic acids, diacetyl) and aroma (sulphurous odours) defects in finished beverages such as beer and wine. The control of bacteria in the brewing process has recently been reviewed by Vriesekoop et al. [17].

In winemaking, certain bacteria may be beneficial, particularly during malolactic fermentation which reduces acidity and results in a smoother tasting wine (Walker [1]). This occurs after the completion of the main yeast fermentation and results in the decarboxylation of L-malic acid to L-lactic acid [18]. This reaction is catalysed by the malolactic enzyme in lactic acid bacteria such as *Oenococcus oeni* which is the dominant bacterial species responsible for the malolactic fermentation. This may occur spontaneously, or may be controlled by addition of pure malolactic starter cultures of *O. oeni*. In Scotch malt whisky fermentations, lactic acid bacteria late in the process are regarded as being beneficial due to imparting acceptable flavour compounds (for example, lactones) that come through in the final distillate [12]. In other distilled spirits, for example, sour-mash bourbons, the growth of lactic acid bacteria is encouraged during certain process steps to depress wort pH and impart desired flavour congeners to the final distillate.

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Further information on the origin of different flavour congeners in the following fermented beverages can be found in the following publications: beer: Boulton and Quain [13] and Priest and Stewart [10]; wine: Fleet [14] and distilled spirits: Walker and Hughes [19], Walker et al. [20], Russell and Stewart [2], and Goodall et al. [21].

# 5. Future Developments in Alcoholic Beverage Fermentations

During alcoholic beverage production, many developments take place involving the yeasts strains employed in fermentations. Research is aimed not only at improving the efficiency of sugar conversion to alcohol, but also in selecting new yeasts to impart desirable flavours and to utilize different carbohydrate raw materials. For Scotch whisky production, Walker et al. [22] have identified desirable characteristics for distilling yeast strains. In brewing and winemaking [23], rapid advances in molecular biology have influenced the application of novel yeast strains in wort and grape juice fermentations, respectively. These developments, notably recombinant DNA technology, can lead to improved fermentation performance and final product quality [23]. Yeast-yeast genetic modification, also called *self-cloning*, represents an attractive technique due to more favourable regulatory issues and consumer acceptability [24]. It is important to note, however, that genetically-modified (GM) *S. cerevisiae* strains for food and beverage fermentations have so far not been widely adopted and are only authorized for use in some countries, but not in Europe [25].

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