Applied Microbiology and Biotechnology (2021) 105:953–973
https://doi.org/10.1007/s00253-020-11080-2

MINI-REVIEW

Thiamine: a key nutrient for yeasts during wine alcoholic fermentation

PWJ Labuschagne[1] · B Divol[1]

Received: 12 October 2020 / Revised: 18 December 2020 / Accepted: 27 December 2020 / Published online: 6 January 2021

Abstract

Alcoholic fermentation is a crucial step of winemaking, during which yeasts convert sugars to alcohol and also produce or biotransform numerous flavour compounds. In this context, nutrients are essential compounds to support yeast growth and ultimately ensure complete fermentation, as well as optimized production of flavour compounds over that of off-flavour compounds. In particular, the vitamin thiamine not only plays an essential cofactor role for several enzymes involved in various metabolic pathways, including those leading to the production of wine-relevant flavour compounds, but also aids yeast survival via thiamine-dependent stress protection functions. Most yeast species are able to both assimilate exogenous thiamine into the cell and synthesize thiamine de novo. However, the mechanism and level of thiamine accumulation depend on several factors. This review provides an in-depth overview of thiamine utilization and metabolism in the model yeast species *Saccharomyces cerevisiae*, as well as the current knowledge on (1) the intracellular functions of thiamine, (2) the balance between and regulation of uptake and synthesis of thiamine and (3) the multitude of factors influencing thiamine availability and utilization. For the latter, a particular emphasis is placed on conditions occurring during wine fermentation. The adequacy of thiamine concentration in grape must to ensure successful fermentation is discussed together with the effect of thiamine concentration on fermentation kinetics and on wine sensory properties. This knowledge may serve as a resource to optimise thiamine concentrations for optimal industrial application of yeasts.

Key points

- *Thiamine uptake is preferred over biosynthesis and is transcriptionally repressed.*
- *Multiple factors affect thiamine synthesis, availability and uptake for wine yeast.*
- *Thiamine availability impacts fermentation kinetics and wine's sensory properties.*

Keywords Thiamine · Yeast nutrition · Wine alcoholic fermentation · *Saccharomyces cerevisiae*

Introduction

Yeasts require certain nutrients to be able to grow, reproduce and maintain metabolism. These nutrients are generally divided into the broad groups of organic carbon sources, nitrogenous compounds, lipids, vitamins and trace elements. Historically, yeast requirements of organic carbon and nitrogenous compounds have been prioritised, because the majority of yeast species are heterotrophic for nitrogen and chemo-organotrophs, thereby requiring organic carbon substrates as a source of energy (Amata 2013; Broach 2012; Joslyn 1951). Other yeast nutrients such as vitamins are also known to significantly affect yeast metabolism and deficiencies thereof may result in cell death (Ough et al. 1989). One such vitamin essential for yeast metabolism is the water-soluble thiamine. This vitamin has been isolated from yeast cultures from as early as 1932 (Begley 1996). Thiamine and its phosphorylated biologically active forms are essential cofactors for the activity of several metabolic enzymes involved in central carbon metabolism pathways such as glycolysis, the pentose phosphate pathway and the tricarboxylic acid cycle (Hohmann and Meacock 1998). Various thiamine-dependent stress protection functions have also been proposed, which may aid yeast cells

✉ B Divol
divol@sun.ac.za

[1] South African Grape and Wine Research Institute, Department of Viticulture and Oenology, Private Bag X1, Matieland 7602, South Africa

to survive under stressful conditions (Kowalska et al. 2012; Wolak et al. 2014). Most yeast species are able to assimilate exogenous thiamine into the cell via an active transport system, but unlike animals, they also possess the metabolic machinery to synthesize their own thiamine (Hohmann and Meacock 1998), albeit at a very large energy cost, as reviewed by Perli et al. (2020). These yeasts generally assimilate all exogenous thiamine before initiating biosynthesis in order to save energy (Suomalainen and Oura 1971; Praekelt et al. 1994). A weak ability of yeast to synthetize their own thiamine under specific environmental conditions, such as those present during wine fermentation, causes yeast to be partially reliant on the availability of exogenous thiamine for maximum growth and metabolism rates (Bataillon et al. 1996; Hohmann and Meacock 1998; Nosaka 2006; Wightman and Meacock 2003).

Under oenological conditions, thiamine deficiency is regarded as a major factor that may cause impaired growth, cell death and subsequently fermentation arrests, whereas increased thiamine availability has been shown to improve yeast cell growth and fermentation rate (Alexandre and Charpentier 1998; Bataillon et al. 1996; Bisson 1999; Ough et al. 1989; Ribéreau-Gayon et al. 2006). Premature arrest of fermentation, generally referred to as a stuck fermentation in the oenological jargon, is a major recurring problem in the wine industry. If yeasts do not acquire certain nutrients such as thiamine early in fermentation, they may fail to grow at a maximum rate, maintain metabolic activity during the stationary phase and ultimately struggle to utilize all available sugars to complete successful alcoholic fermentation (Bataillon et al. 1996; Bisson 1999). The thiamine content of grape must vary extensively (Peynaud and Lafourcade 1958); however, it is reported that yeasts can utilize more thiamine than what grape must contains (Ribéreau-Gayon et al. 2006). In addition, a few hours of contact with even a small population of indigenous yeast species, such as *Kloeckera apiculata*, has proven to deplete thiamine in must (Bataillon et al. 1996). Subsequently, the supplementation of thiamine in musts is a common practice in most winemaking countries in order to sustain cell growth until fermentations are completed. In addition to the positive effect of thiamine availability on fermentation kinetics, it may also alter compounds produced by yeast during fermentation. This includes various aroma compounds, which play an important role in the quality and character of finished wines (Kneen et al. 2011; Müller 2014).

To induce successful fermentation, it is therefore necessary for winemakers to determine whether thiamine and other nutrients are present in sufficient concentrations in must. Literature investigating thiamine and other nutrient deficiencies in grape must is limited. This is most likely due to challenges regarding the analytical determination of such compounds within a complex medium such as grape must, as well as the complexity of factors aside from the grape derived contents itself that affect the amount of specific nutrients present in different grape musts. The latter includes among others, the native yeast population and winery treatments such as juice clarification and duration of skin contact (Houtman and Du Plessis 1986). Apart from the technical issues linked to accurate quantification of thiamine in grape juice, the multifactorial nature of thiamine requirements by yeast under various environmental conditions, the factors involved in the molecular stability of thiamine and regulation of uptake and biosynthesis of yeast have so far not been holistically characterised.

Saccharomyces cerevisiae is being used extensively as a model yeast species in and beyond the realm of oenology. For this reason, research on yeast thiamine requirements has mostly focused on this species. However, other yeast species (indigenous or inoculated) are present and active during winemaking, especially in the first few hours of fermentation. Their thiamine uptake may therefore impact the availability of thiamine for *S. cerevisiae* and ultimately the fermentation success.

This review explores the current knowledge on thiamine utilization and metabolism in *S. cerevisiae*. The biological functions of thiamine, as well as its biosynthesis, uptake and the regulation thereof in yeast are discussed. A particular emphasis is placed on thiamine as a nutrient under oenological conditions. The effect of thiamine availability on oenological fermentation is discussed in terms of fermentation kinetics, as well as the sensorial impact on wine. Furthermore, adequacy of thiamine concentration in grape must to ensure successful fermentation, as well as factors that may impact thiamine availability, uptake and biosynthesis for yeast under oenological conditions are reviewed.

Thiamine: more than just a cofactor

Intracellular forms of thiamine

Thiamine, also referred to as thiamin, aneurin and vitamin B1 (Bhagavan 2002), is a water-soluble B complex vitamin with the following chemical formula: $C_{12}H_{17}N_4OS$. The structure of thiamine is composed of a pyrimidine and a thiazole ring connected by a methylene bridge. The thiazole ring is substituted with methyl and hydroxyethyl side chains (Fig. 1a). Intracellular thiamine consists of free thiamine, thiamine monophosphate, thiamine triphosphate and thiamine pyrophosphate (TPP). The majority of yeast intracellular thiamine is present in the latter form (Makarchikov et al. 2003), which contains a diphosphate functional group connected to the thiazole ring of thiamine (Fig. 1 b).

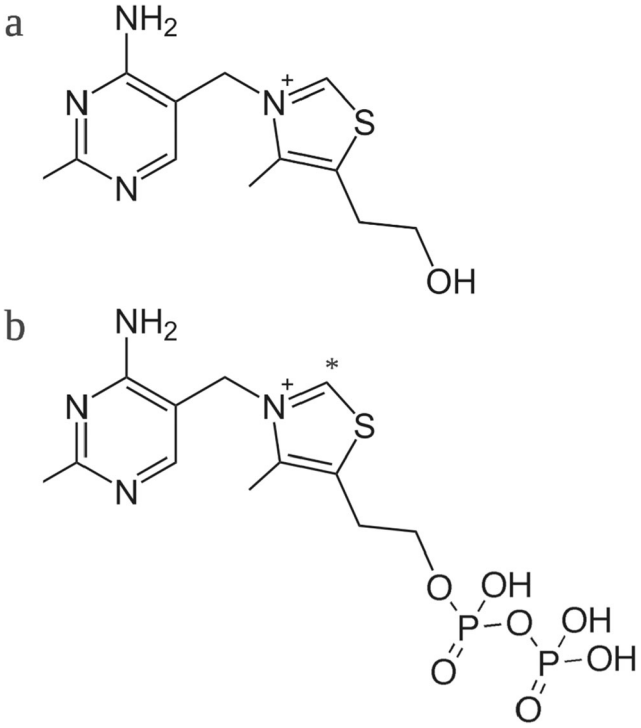

Fig. 1 Chemical structure of **a** thiamine and **b** thiamine pyrophosphate. A single asterisk (*) indicates proton donating reactive carbon 2 of the thiazole ring. Created with BioRender.com

Reaction mechanism of thiamine pyrophosphate

TPP is the most biologically active form of thiamine and fulfils a fundamental function as a cofactor for several enzymes involved in major metabolic pathways including glycolysis, the tricarboxylic acid cycle and the pentose phosphate pathway, as reviewed by Perli et al. (2020). TPP catalyses the reversible decarboxylation reaction for these enzymes by the five following steps: carbon 2 of the thiazole ring of TPP, which is deemed the reactive site of the molecule (Fig. 1 b), may donate a proton to form a nucleophilic carbanion. This carbanion binds to a carbonyl functional group of the substrate molecule with a single carbon–carbon bond. Next, a single bond on the substrate molecule is broken, releasing electrons which causes a double carbon–carbon bond to form between TPP and the substrate and reduces the nitrogen atom of TPP to a neutral state. In a following reverse reaction, the electrons are transferred back towards the substrate, causing a new carbon-hydrogen bond to form on the substrate molecule, leaving again a single bond between TPP and the substrate. The TPP-substrate bond is finally broken, reforming the TPP carbanion and the new substrate-carbonyl compound (Dyda et al. 1993; Malandrinos et al. 2006; Pohl et al. 2004). For TPP to correctly situate in the enzyme cofactor site, the negatively charged phosphate groups of TPP are coordinated by a divalent metal ion such as magnesium or calcium (Schellenberger et al. 1997).

Role of thiamine pyrophosphate as cofactor for central carbon metabolism

The cofactor function of thiamine pyrophosphate extends to several essential enzymes involved in central carbon metabolism, including pyruvate dehydrogenase, pyruvate decarboxylase, acetolactate synthase, α-ketoglutarate dehydrogenase, branched chain α-keto acid dehydrogenase and transketolase (Bunik et al. 2013; Hohmann and Meacock 1998). TPP used to be referred to as co-carboxylase, as it was originally recognized as the coenzyme of pyruvate decarboxylase (Pdc), which catalyses the conversion of pyruvate to acetaldehyde and CO_2 (Trevelyan and Harrison 1954; Muller et al. 1999). Acetaldehyde is thereafter either reduced to ethanol via alcohol dehydrogenase, oxidised via aldehyde dehydrogenase to acetate, which can subsequently serve as an intermediate to form acetyl coenzyme A (acetyl-CoA), or converted to acetoin and subsequently reduced to 2,3-butanediol (Cambon et al. 2006). TPP is also a cofactor for pyruvate dehydrogenase (Pdh), which facilitates oxidative decarboxylation of pyruvate to produce CO_2 and acetyl-CoA (Patel et al., 2014). Acetyl-CoA can be used to synthesize citrate for the tricarboxylic acid cycle (TCA cycle) for the production of adenosine triphosphate (ATP). Therefore, Pdh links glycolysis to the TCA cycle (Remize et al., 2000). Both acetyl-CoA and CoA participate in amino acid, lipid and sulphur metabolism. Subsequently, the availability of TPP can also indirectly alter these processes (Nielsen, 2014).

Transketolase (Tkl) converts fructose-6-phosphate into xylose-5-phosphate and catalyses the production of ribose during the non-oxidative branch of the pentose phosphate pathway (PPP) (Schenk et al. 1998). The PPP is essential for the biosynthesis of intermediates for aromatic amino acid synthesis, as well as generation of reducing equivalents such as the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH), which is involved in maintaining redox balance. The PPP is also involved in the biosynthesis of lipids and the production of ribose-5-phosphate is essential for DNA and RNA synthesis (Dobrowolski and Mirończuk 2020; Rapala-Kozik et al. 2008). Thiamine-dependent Tkl is therefore pivotal for biosynthesis of proteins, lipids, DNA, RNA and alleviation of oxidative stress, which are critical processes for maintaining cell growth and reproduction. Acetolactate synthase (Als) and branched chain α-keto acid dehydrogenase (Bckdh) catalyses the respective biosynthesis and catabolism processes of branched chain amino acids, valine, leucine and isoleucine (Bussey and Umbarger 1969; Dickinson and Dawes 1992). The branched chain amino acids are valuable amino acids which aid in protein synthesis, and many yeasts can utilize these branched chain amino acids as their sole nitrogen sources (Large 1986). The penultimate intermediate for valine synthesis, α-keto isovalerate, is also a precursor for

biosynthesis of the essential vitamin pantothenate, which is indicative of a link between the thiamine and pantothenate metabolisms. The enzyme α-keto acid dehydrogenase (Kdh) catalyses the oxidative decarboxylation of α-ketoglutarate to succinyl-coenzyme A and carbon dioxide (CO_2) in an essential reaction to ensure the functioning of the TCA cycle, which produces ATP (Holz et al. 2011). Kdh is also involved in maintenance of redox balance within the cell (Bunik 2003). A schematic summary of the main pathways and enzymes dependent on thiamine as a cofactor is provided in Fig. 2.

Thiamine-dependent protection against stress

The cofactor function of TPP is critical for facilitating various metabolic processes essential for the survival of yeast cells. This includes maintaining the cellular redox balance under redox stress. It has been demonstrated that TPP-dependent Tkl activity is involved in the cell's oxidative stress response by means of flux into the PPP, to produce necessary the NADPH and glutathione to mitigate oxidative stress (Kowalska et al. 2012; Stincone et al. 2015). Thiamine deficiency is indeed regarded as a major cause of systemic oxidative stress (Wang et al. 2005). Several additional stress protection functions of TPP, in addition to its cofactor function have been proposed. TPP has shown to serve an antioxidant function via interaction with free radicals and hydroperoxides, yielding thiochrome and thiamine disulphide. This oxidation effect is hypothesised to be a result of the transfer of two protons from the pyrimidine ring and one proton from the thiazole ring to these reactive molecules (Lukienko et al. 2000). A study using *S. cerevisiae* revealed that the presence of thiamine did indeed decrease the free radical levels, as well as protein oxidation under various stress conditions (Wolak et al. 2014).

Another study investigating thiamine triphosphate (TTP) assimilation in response to amino acid deficiency suggested the first physiological role of thiamine triphosphate (TTP) to be a universal signalling molecule under stress conditions (Lakaye et al. 2004). An additional link between thiamine and stress resistance involves the protein Thi4, which is known to facilitate thiazole synthesis during thiamine biosynthesis. In addition to its role in thiamine biosynthesis, Thi4 has shown to be essential to maintain adequate oxygen consumption of cells, which prevents formation of high levels of reactive oxygen species, which may become toxic for yeast. Increased levels of Thi4 have also shown to increase mitochondrial stability under heat stress (Medina-Silva et al., 2005). Additionally, Thi4 may assist in the repair of mitochondrial DNA damage following ultraviolet radiation (Machado et al. 1997). The synthesis of this multifunctional protein therefore provides a link between thiamine synthesis and mitochondrial stress tolerance.

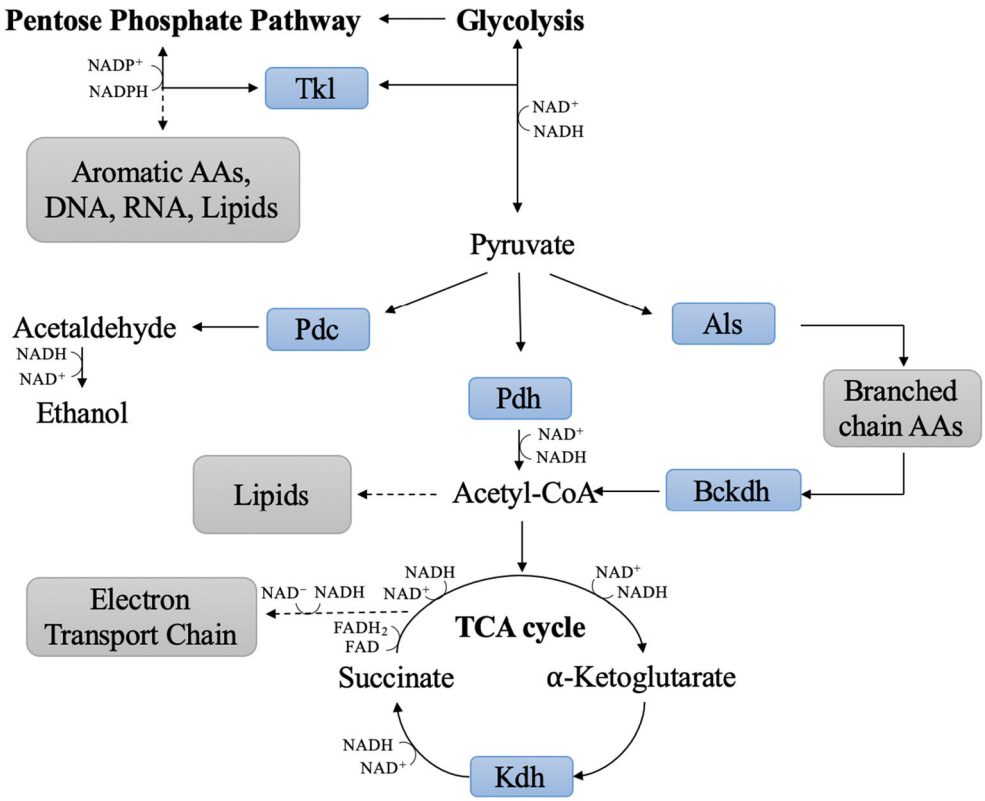

Fig. 2 Thiamine-dependent enzymes in yeast metabolism. Thiamine-dependent enzymes (blue), central metabolic pathways (black), peripheral metabolic pathways (grey). Pdh, pyruvate dehydrogenase; Pdc, pyruvate decarboxylase; Als, acetolactate synthase; Tkl, transketolase; Kdh, α-ketoglutarate dehydrogenase; Bckdh, branched chain α-keto acid dehydrogenase; TCA cycle, tricarboxylic acid cycle; AAs, amino acids. Redox reactions involving the oxidized and reduced forms of nicotinamide adenine dinucleotide (NAD^+/NADH), nicotinamide adenine dinucleotide phosphate ($NADP^+$/NADPH) and flavin adenine dinucleotide (FAD^+/$FADH_2$) are displayed

Biosynthesis and uptake mechanisms

Thiamine biosynthesis and origins of thiamine precursors

Thiamine biosynthesis is achieved in yeasts through the initial synthesis of thiazole and pyrimidine, which are subsequently phosphorylated to 5-(2-hydroxyethyl)-4-methylthiazole phosphate (HET-P) and 4-amino-5-hydroxymethyl-2-methylpyrimidine diphosphate (HMP-PP), respectively, and amalgamated to form thiamine monophosphate (TP) (Estramareix 1996; Nosaka 2006). Biosynthesis of thiazole requires 2-pentulose-5-phosphate, either xylulose-5-phosphate or ribulose-5-phosphate, which is provided by the PPP and glycine (White and Spenser 1982). Cysteine is required to serve as a sulphur donor. The exact process for thiazole biosynthesis is not fully understood, but a mechanism has been suggested where NAD^+ serves as a substrate for the Thi4 enzyme, which in turn catalyses the production of adenosine diphosphate-ribulose and release of nicotinamide (Chatterjee et al. 2007). In a subsequent step, a decarboxylation and dehydration reaction forms thiazole phosphate, which can combine with the pyrimidine subunit to form thiamine. Yeasts also possess the ability to assimilate external thiazole, in the form of hydroxyethylthiazole, which can subsequently be phosphorylated intracellularly (Nosaka et al. 1994). Thiazole is assimilated via diffusion and trapped within the cell cytoplasm by phosphorylation (Iwashima et al. 1986).

The substrates for pyrimidine synthesis include histidine and pyridoxal-5-phosphate, the latter derived from ribose 5-phosphate and glutamine glyceraldehyde 3-phosphate (Hanes et al. 2008; Tazuya et al. 1995a, 1995b). Yeasts can also acquire exogenous pyrimidine in the form of hydroxymethylpyrimidine (HMP) by utilizing the same active transport as for free exogenous thiamine. However, a phenomenon where assimilated HMP is expelled from the cell due to excessive uptake has been documented for *Saccharomyces cerevisiae*, whereas this in not the case for free thiamine (Iwashima et al. 1990). Pyrimidine is finally subjected to two phosphorylation steps, catalysed by pyrimidine kinase and pyrimidine phosphate kinase, to form pyrimidine diphosphate, before combining with thiazole phosphate to produce TP (Llorente et al., 1999). More recently, a new thiamine salvage pathway from base degraded forms of thiamine has been reported. The base degraded thiamine analogues, such as formylaminopyrimidine, may be transported into the cell via a ThiXYZ active transport system, deformylated by an aminohydrolase, and hydrolyzed to HMP for thiamine biosynthesis (Jenkins et al. 2007). A similar thiamine salvage reaction by the hydrolysation of thiamine antimetabolites, pyrithiamine and oxythiamine to form HMP has been demonstrated for *S. cerevisiae* (Onozuka et al. 2008).

Exogenous thiamine uptake: a less energy demanding alternative to biosynthesis

Yeasts favour the assimilation of exogenous thiamine before initiating thiamine biosynthesis in order to conserve energy. This may be attributed to the fact that thiamine biosynthesis requires several more phosphorylation reactions and therefore more energy in the form of ATP compared to exogenous thiamine uptake (Suomalainen and Oura 1971; Praekelt et al. 1994). In addition, two suicide enzymes (Thi4 and Thi5) are involved in thiamine biosynthesis. Therefore, two entire proteins need to be synthesized and degraded for each thiamine molecule produced, making this process energetically expensive (Chatterjee et al. 2011; Wightman and Meacock 2003).

There are two main proteins involved in thiamine uptake. The first is a thiamine-repressible acid phosphatase (Pho3) located in the cell periplasm which serves to dephosphorylate thiamine phosphates before active uptake of the free thiamine (Enjo et al. 1997; Nosaka et al. 1989). This active uptake into the cytoplasm is facilitated by a second membrane-bound transporter protein (Thi7/Thi10), which is able to bind free thiamine (Iwashima et al. 1979; Singleton 1997). This transporter protein presumably recognises the pyrimidine moiety of thiamine since pyrimidine is also actively transported into cells, unlike free thiazole (Iwashima and Nose 1976). The K_m value, an indicator of the affinity of the transporter protein for the transported substrate, of thiamine is measured at 0.18 mM, which is very similar to the dissociation constant K_d, for thiamine binding to the membrane protein (Iwashima et al. 1979). Two additional membrane proteins, Thi71 and Thi72, have more recently shown to facilitate transport of thiamine (Enjo et al. 1997; Mojzita and Hohmann 2006). However, Thi7 facilitates the majority of thiamine uptake and may be exclusively responsible for transport at low exogenous concentrations, whereas Thi71 and Thi72 are low affinity and low capacity transport systems. Another uncharacterised protein, Thi73, is known to be upregulated during thiamine deficiency and has been proposed as an additional transporter, but this is yet to be established (Mojzita and Hohmann 2006; Nosaka et al. 2005). Once thiamine is assimilated into the cell, yeasts do not possess the ability to phosphorylate TP directly to the biologically active TPP. TP is first dephosphorylated to free thiamine, which is then subjected to a single-step diphosphorylation to form TPP (Nosaka et al. 1993). TPP functions not only in the cytosol, but also in the mitochondrion of cells, for example as a cofactor for α-ketoglutarate dehydrogenase involved in the TCA cycle. An energy-dependent mitochondrial membrane protein, Tpc1, facilitates transport of cytosolic TPP into the mitochondria (Marobbio et al. 2002). A schematic summary of thiamine biosynthesis and uptake is provided in Fig. 3.

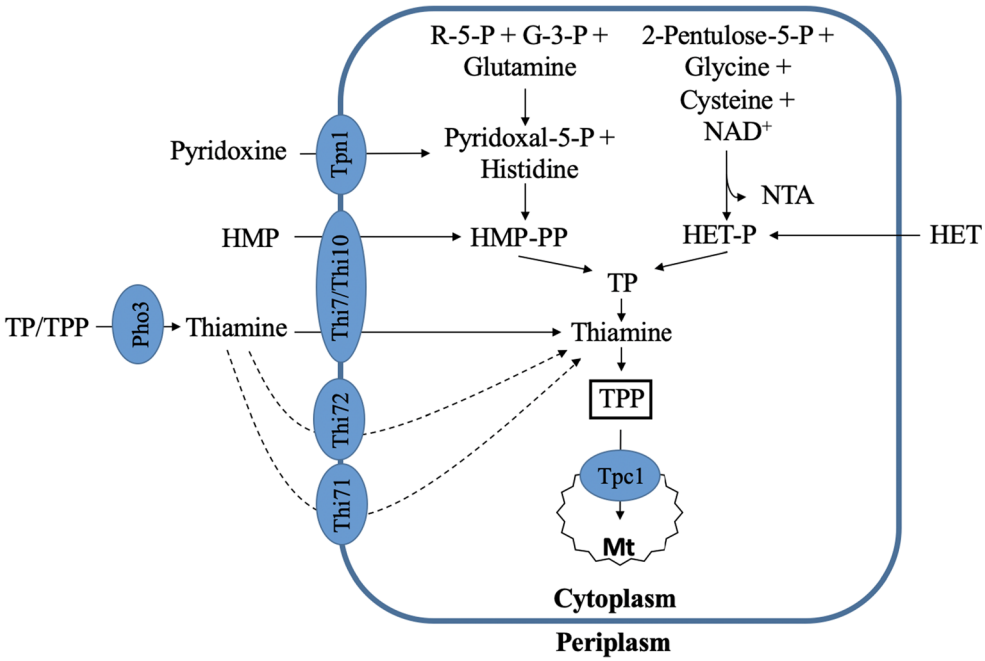

Fig. 3 Thiamine biosynthesis and uptake in yeast. G-3-P, glyceraldehyde 3-phosphate; R-5-P, ribose 5-phosphate; HMP, hydroxymethylpyrimidine; HMP-PP, hydroxymethylpyrimidine pyrophosphate; HET, hydroxyethylthiazole; HET-P, hydroxyethylthiazole phosphate; NAD^+, nicotinamide adenine dinucleotide; NTA, nicotinamide; TP, thiamine monophosphate; TPP, thiamine pyrophosphate; Mt, mitochondria

The active, early and rapid nature of exogenous thiamine transport

Exogenous thiamine assimilation by yeast is an energy-dependent carrier-mediated process in which thiamine is retained against a concentration gradient. *S. cerevisiae* accumulates thiamine by an irreversible active transport system, which allows for the accumulation of intracellular thiamine up to approximately 10,000 times the extracellular concentration (Iwashima et al. 1973; Nakamura et al. 1982). However, thiamine transport by *S. cerevisiae* does exhibit saturation kinetics, as high exogenous thiamine concentrations (40 μM) have shown to result in a saturated intracellular concentration of 1600 pmol/10^7 cells, roughly 180 times the intracellular concentration of cells grown without thiamine. Assimilation causes a rapid rise of intracellular thiamine concentration, at which point transport and biosynthesis is repressed, and a subsequent gradual decrease of intracellular thiamine concentration is observed as a result of dilution during growth (Praekelt et al. 1994).

S. cerevisiae starts to accumulate thiamine within 15 min following inoculation into a medium-containing glucose. This short lag period of thiamine transport is attributed to the lack of sufficient energy supply to maintain the thiamine transport rate at this early stage. Indeed, pre-incubation of cells in media with glucose has been shown to enable the cells to assimilate thiamine without any appreciable lag phase, even in the absence of glucose (Ziro 1955). Maximum thiamine accumulation levels are observed before appreciable growth of cells occurs, demonstrating the priority for cells to utilize energy for accumulation during early stages after inoculation (Nakamura et al. 1982). The lag before initiation of thiamine uptake was found to be independent of the thiamine and oxygen concentration present in the medium and solely dependent on glucose concentration. Praekelt et al. (1994) reported that a low concentration of exogenous thiamine (1 μM) was completely assimilated from a molasses medium within 30 min and a high level of exogenous thiamine (40 μM) was assimilated within 2 h. Moreover, an early study determined that for *S. cerevisiae* incubated in 0.1 M phosphate buffer, pH 5.0, at 27 °C, under both aerobic- and anaerobic conditions; the concentration of thiamine assimilated by cells was equal to the concentration of thiamine lost from the medium. This demonstrated that the thiamine removed from the medium was neither broken down nor converted, but entirely accumulated by the yeast cells (Ziro 1955).

Factors that affect thiamine uptake and biosynthesis: a lasting impact of preculture conditions

Transport of exogenous thiamine across the cell membrane has proven to be pH and temperature sensitive, with the transporter operating optimally at a pH of 4.5 at both 27 °C and 37 °C. The rate of exogenous thiamine uptake by *S. cerevisiae* is roughly proportional to the cell concentration present (Bataillon et al. 1996), but independent of the exogenous thiamine concentration, since thiamine is transported via an active transport system (Hucker et al. 2016; Iwashima et al. 1973; Ziro 1955). However, exogenous thiamine assimilation is known to be inhibited when cells are first precultured in thiamine-rich media with maximum assimilation rates observed for cells pre-cultured in thiamine-limited media before inoculation into another medium (Bataillon et al. 1996; Iwashima and Nose, 1976). This inhibition effect can be

attributed to the fast uptake of thiamine and subsequent sustained repression of thiamine biosynthesis and transport genes as a result of increased TPP levels (Praekelt et al. 1994; Nosaka 2006). Indeed, it has been observed that cells pre-cultured in excess thiamine displayed an absence of thiamine transporter proteins and that intracellular accumulation of thiamine induces endocytosis of the Thi7 thiamine transporter (Iwashima et al. 1979; Savocco et al. 2019).

Pre-culture harvest time significantly affects thiamine assimilation. Cells harvested near the end of the exponential growth phase of the yeast population, 15 h after inoculation, display maximal thiamine uptake (Bataillon et al. 1996). Thiamine is assimilated at a maximum rate for actively growing cells (Iwashima and Nose 1976). Indeed, it has been reported that the activity of the thiamine-binding protein and thiamine transport protein in *S. cerevisiae* is maximal in the early logarithmic phase of growth (Iwashima et al. 1979). Under thiamine-deficient conditions, the thiamine biosynthesis rate too has been determined to be maximal during early logarithmic phase of growth. However, the same study also recorded the activation of thiamine biosynthesis during late logarithmic phase, after the initially supplemented exogenous thiamine was depleted. This confirms the requirement of cells to maintain adequate thiamine levels, either to sustain metabolic enzyme activity and/or as a function of thiamine-dependent protection against stress factors later in the fermentation process (Kowalska et al. 2012).

Additional factors that influence thiamine uptake and biosynthesis include dissolved oxygen concentration, level of yeast agitation in growth media and the presence of pyridoxine and short-chain fatty acids. These factors are discussed in a separate paragraph below with a particular focus placed on wine fermentation conditions.

Regulation of thiamine biosynthesis and uptake

Thiamine biosynthesis and uptake are mainly regulated via transcriptional repression. Evidence of post-transcriptional regulation mechanisms such as riboswitches, which are present in some yeast species, have not been reported for *S. cerevisiae* (Cressina et al. 2011). Transcriptional repression has shown to be initiated by small changes in intracellular thiamine levels. The presence of intracellular thiamine causes the expression of all annotated genes encoding proteins involved in biosynthesis (*THI80*, *THI4*, *THI5* and *THI6*) (Praekelt et al. 1994; Nosaka et al. 1994) and uptake (*THI7* and *PHO3*) (Singleton 1997) of thiamine to be repressed. Activation of *THI4* involved in thiazole synthesis is for instance initiated when the intracellular thiamine concentration descends below 20 pmol/10^7 cells. This gene is completely repressed when an exogenous thiamine concentration of 1 μM is available, but displays a high level of constitutive expression when there is no exogenous thiamine available (Praekelt et al. 1994). A decrease in available exogenous thiamine for yeast cells during fermentation is also known to initiate a high level of expression for other *THI* genes. Indeed, conditions where exogenous thiamine is absent have shown to trigger transcript level increases of between 10- and 90-fold for the *THI* genes involved in thiamine biosynthesis and uptake in *S. cerevisiae* (Nosaka et al. 2005).

THI genes are positively regulated by three genes, namely *THI2* (Nishimura et al. 1992b), *THI3* (Nishimura et al. 1992a) and *PDC2* (Burrows et al. 2000). The expression of the *THI* genes is therefore terminated when these regulator genes are not present or expressed. Other genes involved in thiamine biosynthesis, for example *SNZ2/3* genes involved in pyridoxal-5-phosphate synthesis, are also regulated via *THI2* and *THI3* (Rodríguez-Navarro et al. 2002). Thi3 is a TPP-binding protein which serves as a sensor for TPP, which subsequently induces transcriptional modulation. This was demonstrated using mutant cells that possess Thi3 proteins lacking the ability to bind to TPP. For these cells, the *THI* genes were expressed irrespective of the thiamine concentration present, thereby confirming that TPP binding to Thi3 causes transcriptional regulation observed for normal cells. The deletion of the *THI3* gene has proven to decrease activation of *THI2* and *THI3* promoter activities, demonstrating an auto-regulatory role of *THI3*, which may in part account for the substantial differences in expression of *THI* genes in conditions of varying thiamine availability. Additionally, Thi2 and Thi3 have shown to bind to one another, stimulating the expression of the *THI* genes as a complex. This binding activity is decreased with increased levels of thiamine, believed to be a result of increased TPP which binds to Thi3 and hinders formation of this Thi2/Thi3 complex. The expression levels of *THI2* and *THI3* increase dramatically under thiamine-deficient conditions (Nosaka et al. 2005). *PDC2* is also involved in the expression of the *THI* genes, as well as expression of structural pyruvate decarboxylase genes (Raghuram et al. 1994), providing a possible link between the regulation of TPP-dependent enzyme synthesis as well as synthesis of TPP itself. An interaction between Pdc2 and Thi3 has also been documented and has shown to be stronger and more sensitive to thiamine deficiency than the earlier mentioned interaction between Thi2 and Thi3. This interaction catalyses a conformational change in Pdc2, making full transactivation activity of the protein possible and effectuating upregulation of the *THI* genes during thiamine deficiency. It is postulated that Thi2, Thi3 and Pdc2 may together form a complex that activates the expression of *THI* genes and that the formation of this complex is disrupted by TPP, which binds Thi3 (Brion et al. 2014; Nosaka et al., 2005).

It has subsequently been determined that the intracellular NAD^+ concentration is also involved in the regulation of several *THI* genes in *S. cerevisiae*. This regulation is facilitated by the NAD^+-dependent histone deacetylases, Hst1 and Sir2,

which may repress *THI* genes as transcription factors which are independent of the Thi2/Thi3/Pdc2 regulatory system. Hst1 and Sir2 are supposedly bound upstream of the Thi2/Thi3/Pdc2-binding site as the respective protein complexes Hst1/Rfm1/Sum1 and Sir2/Sir3/Sir4 and impose regulation of *THI* genes by local histone deacetylation activity triggered by low NAD^+ concentration. The peak association for each complex occurs at the same gene region, suggesting that these complexes are alternative repression complexes that do not bind at the same time. Under conditions of decreased intracellular concentration of NAD^+, Hst1 deacetylation activity is reduced by bound Hst1/Rfm1/Sum1or Sir2/Sir3/Sir4 complexes, which promotes increased *THI* gene expression (Li et al., 2010). Full expression of *THI* genes is therefore a function of both low thiamine and low NAD^+ concentrations and full repression of *THI* genes are observed at high thiamine and high NAD^+ concentrations. The mechanisms of *THI* gene regulation driven by thiamine and NAD^+ concentrations are detailed in Fig. 4.

Thiamine as nutrient for alcoholic fermentation

Adequacy of thiamine concentration in grape must

The thiamine concentration present in grapes increases during grape maturation, particularly during ripening (Peynaud and Lafourcade 1958). It has been reported that the thiamine concentration of grapes continues to increase even after sugar formation in the berry ceases, which likely indicates a continuation of the photosynthetic action on thiamine accumulation of berries until the time of harvest (Daudt and Parizzi 1995). Literature on specific viticultural practices and thiamine content of resulting harvested grapes is lacking. It is, however, known that the thiamine content of must varies greatly. Studies investigating thiamine content of several musts of various origin, cultivar and vintage, displayed thiamine concentrations between 120 and 650 μg/L directly after pressing. Across all studies, white cultivars average lower thiamine concentrations compared to red cultivars. The results of these studies are summarized in Table 1.

The variations in thiamine concentrations between studies are likely due to environmental and varietal influences, but the use of different quantification methods and the accuracy of these methods may also contribute to the variance observed.

The methods employed in these thiamine quantification studies rely either on oxidation to the thiochrome and subsequent fluorescence detection or microbiological assays (Table 1) that monitor the growth of microorganisms such as *Lactobacillus fermentum* in response to thiamine concentration. These methods are still in use today as a result of their relative accuracy and ease of use, although various other high-performing methodologies have since been developed. For a detailed review, refer to Edwards et al. (2017). When comparing these methods, fluorescence detection allows for direct quantification of various thiamine phosphate forms, whereas microbiological assays are subject to the utilization of these forms by the microorganism employed.

S. cerevisiae strains are reportedly able to assimilate up to 800 μg/L of thiamine during fermentation (Ribéreau-Gayon et al. 2006), which is even more than what the grape musts with the highest concentration of thiamine in the studies mentioned above, contained. The average thiamine concentration in grape must, as well as reported levels of thiamine assimilation by *S. cerevisiae*, are broad estimations and may be altered by various factors that will be reviewed below. Yeasts preferentially assimilate thiamine at the start of wine fermentation and all thiamine is assimilated within 6 h after inoculation of *S. cerevisiae* (Bataillon et al. 1996). As a result, the addition of thiamine hydrochloride (thiamine HCl) or complex nutrients that contain thiamine to grape musts is commonly practiced in numerous cellars. Thiamine HCl dissociates in solution to form the free thiamine cation, referred to simply as thiamine, and two chloride anions (Voelker et al. 2018). Thiamine HCl has a molecular weight of 337.3 g/mol, whereas free thiamine has a molecular weight of 265.4 g/mol. Therefore, an addition of one unit of thiamine HCl to must equals 0.787 units of free thiamine available for yeast. The addition of thiamine to must for winemaking purposes is legal in the European Union and South Africa at a concentration of 600 μg/L thiamine HCl, according to the International Code of Oenological Practices 2020 Issue (OIV 2020).

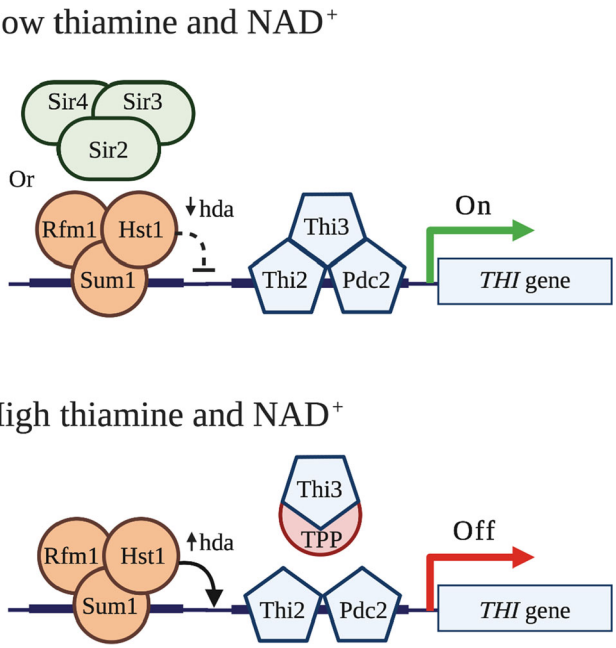

Fig. 4 Hypothetical model of the *THI* gene regulatory system of *S. cerevisiae* (based on models of Li et al., 2010 and Nosaka, 2006). Created with BioRender.com

Table 1 Thiamine concentration of grape musts

Type of wine (cultivar)	Average concentration (μg/L)	Quantification method	Reference
Red: Piros tramini, Kekfrankos, Zweigelt, Biborkadarka, Rubintos White: Chasselas, Hárslevelű, Olaszrizling, Rizlingszilváni, Füszeres tramini	Red - 198 White - 138	Microbiological assay	Juhász et al. (1987)
Red: Aramon, Carignan, Alicante Bouschet, Cinsault White: Terret Blanc, Carignan Blanc, Macabeo	Red - 246 White - 185	Fluorescence detection	Ournac and Flanzy (1958)
Red: Tinta Madeira, Xeres, Zinfandel White: Palomino, White Riesling	Red - 500 White - 340	Fluorescence detection	Hall et al. (1956)
Various red and white cultivars	160–450	Microbiological assay	Peynaud and Lafourcade (1958); Ribéreau-Gayon et al. (1975)

Factors influencing the availability of thiamine for *S. cerevisiae* during winemaking

Indigenous yeast and fungi

A deficiency of thiamine (considered to be < 250 μg/L) in grapes and grape musts may be caused by growth of filamentous fungi such as *Botrytis cinerea* (Dittrich and Sponholz 1975) on grapes before harvest, or by growth of native contaminant yeast species such as *Kloeckera apiculata*, *Metschnikowia pulcherrima* and *Candida stellata*, which are present in must at high population levels (ordinarily 10^3–10^5 viable cells/ml) before inoculation occurs in cellars. Indeed, the thiamine consumption of *K. apiculata*, which may make up 90% of the total native yeast present in must (Schütz and Gafner 1993), commences at a faster rate than for the same amount of viable *S. cerevisiae* cells in grape must (Bataillon et al. 1996). Consequently, *K. apiculata* is able to effectively deplete thiamine from must after a few hours of growth, even when present at very low cell concentrations. Additionally, *K. apiculata* can accumulate an amount of exogenous thiamine up to one-tenth of its dry cell weight (Kawasaki et al. 1967). This rapid accumulation of large portions of available thiamine by native yeast species at the start of alcoholic fermentation influences the availability thereof for subsequent assimilation of inoculated *S. cerevisiae*. Co- or sequential inoculation regimes may cause a similar problem, where inoculated non-*Saccharomyces* yeasts assimilate available thiamine (Julies 2019). This may cause a lack of thiamine to sustain growth of subsequently inoculated *S. cerevisiae*, which is employed specifically to complete fermentation. Indeed, competition for vitamins such as thiamine has been proposed to cause stuck fermentation of *Zygoascus meyerae*/*S. cerevisiae* sequential cultures in synthetic grape must (Rollero et al. 2018).

Sulphur dioxide

Some winemaking practices may affect the thiamine levels of must before inoculation occurs. As mentioned, growth of native yeasts in must may deplete available thiamine. Therefore, the time elapsed from initial crushing of grapes to final inoculation of the must, as well as antimicrobial measures taken during this period such as sulphur dioxide addition, may affect the concentration of thiamine assimilated by native yeasts. While sulphur dioxide (SO_2) may inhibit growth of native yeast and other contaminant organisms that assimilate available thiamine, it is also known to cause degradation of available thiamine. Thiamine is cleaved at the methylene bridge in a linear fashion dependent on SO_2 concentration (Leichter and Joslyn 1969; Dwivedi and Arnold 1973). A decrease in free thiamine as a result of high SO_2 additions to must or extended must storage periods in the presence of SO_2 may attribute to sluggish fermentation (Boulton et al. 1996). It has been previously observed that 8 days after sulfiting grape must to a level of 0.5 g/L, half of the initial thiamine concentration was destroyed and all thiamine was destroyed after a month in these conditions (Peynaud and Lafourcade 1958). Nevertheless, the abovementioned SO_2 concentration is higher than what would typically be supplemented to must and the effect of more standard SO_2 concentrations still needs to be investigated.

Temperature and pH

The molecular stability of thiamine may be compromised by various other factors. The structural integrity of thiamine is known to be susceptible to degradation by particularly high temperatures and high pH. Destruction of thiamine by high pH and temperature can result from simple hydrolytic cleavage or oxidation by available hydroxyl groups. Thiamine degradation generally results in cleavage of its pyrimidine and

thiazole constituents, by separation at the methylene bridge that connects these segments. Thiamine is chemically stable at an acidic pH but is unstable in alkaline solutions and a marked increase in the rate of thiamine degradation has been observed at pH values above 6 (Mauri et al. 2007; Tanphaichitr 1999). The proton on the carbon 2 of the thiazole ring is acidic and may dissociate at pH values above 5.5 to form a carbanion which may bind to carbonyl groups. Thiamine also becomes unstable at increasing temperatures, but stable from 0 °C up to at least 25 °C (Dwivedi and Arnold 1973; Haddad and Loewenstein 1983). Thiamine suspensions stored at both 4 °C and 25 °C exhibit less than 5% loss of an original concentration of 100 g/L for up to 91 days (Ensom and Decarie 2005). Grape must pH usually ranges between 3.0 and 4.5, which is favourable for thiamine stability. The recommended maximum fermentation temperature for winemaking is 25 °C, but in some wine-producing regions, the temperatures reached during grape pressing may reach up to 40 °C (Moreno and Peinado 2012). It is, therefore, unlikely that these temperature levels would cause significant thiamine destruction over such a relatively short time period.

Thiaminases

Thiaminase enzymes have shown to break down thiamine in a similar fashion. There are two known types of thiaminases, thiaminase I and thiaminase II, both of which require electron donation from nucleophiles. Thiaminase II utilizes water as its nucleophile, while thiaminase I may utilize various nucleophiles, including nicotinic acid, amino acids such as lysine and cysteine, sulphhydryl compounds such as dithiothreitol and β-mercaptoethanol and organic bases such as pyridine and quinoline. Thiaminase I is present in certain plants and microorganisms, whereas thiaminase II has only been isolated from microorganisms (Campobasso et al. 1998). Some bacterial species of *Bacillus* and *Clostridium sporogenes*, as well as yeast species *Candida aneurinolytica* and *Trichosporon aneurinolyticum* have displayed thiaminase activity (Shimazono and Katsura 1965). Literature investigating the presence and effect of thiaminases in grape must is absent, although higher activity of thiaminases have been reported in grapes compared to other fruit (Okonji and Agboola 2014). Its impact on thiamine levels of grape must for yeast utilization thus warrants investigation.

The pyrimidine and thiazole products of thiamine cleavage can be assimilated by yeast through the salvage thiamine biosynthesis pathway, but as stated above, exogenous accumulation of thiamine is preferred over biosynthesis of thiamine to conserve energy. It has recently been suggested that a thiaminase II is involved predominantly in the pyrimidine salvage pathway rather than in thiamine degradation. Studies on the substrate specificity of thiaminase II have revealed that it only catalyses the hydrolysis of thiamine and not the synthesised biologically active phosphorylated forms of thiamine. Additionally, the hydrolysis of base degraded forms of thiamine, such as aminopyrimidine, is catalysed approximately 100-fold faster than thiamine (Jenkins et al. 2007). It has been reported that the Thi20 enzyme produced by *S. cerevisiae*, which catalyses the phosphorylation of hydroxymethylpyrimidine (HMP) during thiamine biosynthesis, serves a dual role as a thiaminase II (Haas et al. 2005). Indeed, Thi20 participates in the mentioned thiamine salvage reaction by the hydrolysation of thiamine antimetabolites, such as pyrithiamine and oxythiamine to form HMP, which is subsequently phosphorylated by the same enzyme to yield the thiamine precursor HMP pyrophosphate (Onozuka et al. 2008). The physiological function of the thiaminase II activity of Thi20 is supported by the fact that the expression of the *THI20* gene is only activated in response to thiamine deprivation (Llorente et al. 1999).

Thiamine analogues

A high concentration of thiamine analogues, such as oxythiamine, pyrithiamine, chloroethylthiamine and dimethialium may affect thiamine uptake and biosynthesis of yeast. Each may inhibit thiamine uptake to a different degree. For *S. cerevisiae*, a ratio of analogue to thiamine of 1:1 resulted in the inhibition of thiamine uptake of between 38 and 43%. This is because the thiamine transport system of *S. cerevisiae* displays structural specificity, not only for thiamine but also for thiamine analogue compounds. *S. cerevisiae* mutants defective in thiamine transport, for instance, display greater resistance to the thiamine analogue, pyrithiamine (Iwashima et al. 1973, 1975). Assimilated thiamine antimetabolites such as oxythiamine is phosphorylated intracellularly and may bind to and inhibit TPP-dependent enzymes (Tylicki et al. 2005). The thiamine antimetabolites pyrithiamine and oxythiamine showed to both inhibit the binding and transport of thiamine in *S. cerevisiae*, but notably thiamine phosphates such as 2-methyl-4-amino-5-hydroxymethylpyrimidine and O-benzoylthiaminedisulfide did not cause inhibition (Iwashima et al. 1979). A recent study observed that oxythiamine presence stimulates Thi7 endocytosis, similar to what would be expected for thiamine presence, which would account for the observed transport inhibition (Savocco et al. 2019). Pyrithiamine has not only shown to cause a reduction of thiamine transport but also inhibition of pyrophosphokinase, which facilitates phosphorylation of thiamine to biologically active TPP (Iwashima et al. 1975; Nosaka et al. 1993).

Thiamine antimetabolites

Some polyphenolic compounds known to be present in grape must have proven to exhibit antimetabolic properties when

interacting with thiamine, which may lead to thiamine deficiency. These compounds include tannins and catechins, composed of hydroxyl-substituted rings which may be oxidized to form quinones. These quinones may oxidize thiamine, forming thiamine disulfide (Panijpan and Ratanaubolchai 1980). This reaction is, however, dependent on pH and degradation of thiamine is minimal at pH 5.5, but increases rapidly corresponding with an increase in pH (Yang and Pratt 1984). The low pH of grape must mitigates this issue under most typical oenological conditions. Oxygenating species are also known to accelerate thiamine loss in a pH-dependent fashion. This reaction entails the conversion of thiamine into oxythiamine, thiochrome or thiamine dimers by formation of a disulfide bond (Dwivedi and Arnold 1973). Cysteine may also participate in thiamine loss through the formation of mixed disulfides. However, other amino acids and peptides seem to protect thiamine from degradation, which would likely be the case in grape must.

Oxygen availability

Some factors may specifically affect yeast thiamine transport and biosynthesis during fermentation. One of these include oxygen availability. High oxygen availability is known to be associated with increased cell viability, metabolism and faster fermentation rates (Aceituno et al. 2012; Rosenfeld et al. 2003). This increased metabolism and ATP production may result in increased thiamine transport and biosynthesis. A parallelism between the assimilated thiamine concentration and the rate of oxygen uptake has been reported (Ziro 1955). Anaerobic conditions, which normally occur as a result of fermentation under oenological conditions, may impose a comprehensive metabolic response on yeast, which could affect thiamine metabolism. Low oxygen concentrations have shown to activate post-transcriptional upregulation of genes involved in the TCA cycle and mitochondrial translation machinery for *S. cerevisiae*. This may alter ATP production and thiamine metabolism (Rintala et al. 2009). Another study observed that anaerobic conditions causes upregulation of a post-translational mechanism for thiamine synthesis by the filamentous fungus, *Aspergillus nidulans* (Shimizu et al., 2016). For *S. cerevisiae*, activity of HMP biosynthesis was reported to depend on oxygen availability (Wightman and Meacock 2003). Gene deletion studies suggest that *S. cerevisiae* is nevertheless able to synthesize HMP under anaerobic conditions using an alternative pathway which does not utilize pyridoxine and histidine as intermediates. This pathway is yet to be determined. The pathway for thiazole biosynthesis is evidently unaffected by oxygen concentration (Tanaka et al. 2000). A study by Hucker et al. (2016) reported no influence of dissolved oxygen concentration on thiamine uptake of yeast during fermentations in simulated brewing wort, but the preculture of cells on malt extract agar which may contain thiamine probably affected this result. It is clear that research regarding the effect of oxygen availability on the regulation of thiamine uptake and biosynthesis is lacking, especially under oenological conditions (Nosaka et al., 2005; Hohmann and Meacock 1998).

Agitation

The effect of agitation on thiamine assimilation under oenological conditions has also not been directly addressed in literature. One study did show that thiamine accumulation in yeasts during fermentation in 250-mL Erlenmeyer flasks with continuous shaking was more than two-fold greater than in 5-L fermenters (Stieglitz et al. 1974). Leakage of small concentrations of intracellular thiamine from cells containing high levels of thiamine has been observed after vigorous agitation, but the addition of glucose terminated this leakage, which suggests that sufficient generation of ATP is required to maintain accumulated thiamine in the cell (Ziro 1955).

Short-chain fatty acids

Under anaerobic conditions, short-chain fatty acids (SCFA) and medium-chain fatty acids (MCFA) accumulate in the yeast during fermentation and may become toxic for cells (Bardi et al. 1999). An increase in SCFA may inhibit the action of glycolytic enzymes, including Pdh and Pdc, as well as inhibit phosphate uptake in yeast (Samson et al. 1955; Neal et al. 1965). In addition, SCFA may inhibit thiamine uptake. The addition of 20 mM of the SCFA caproate may for instance trigger a rapid efflux of thiamine from *S. cerevisiae* cells (Iwashima et al., 1973). An increase in SCFA causes a reduction in oxygen consumption rate and subsequently slower glycolytic metabolism of yeast, but the substantial suppression of thiamine uptake cannot not only be ascribed to this, as it was observed that SCFA inhibited anaerobic thiamine uptake without a substantial decrease in the concentration of intracellular ATP. It is now clear that SCFA may predominantly affect the cell membrane by preventing the utilization of ATP for the transport of thiamine, as well as preventing thiamine's coupling to the transport protein at higher SCFA concentrations. The level of inhibition of thiamine uptake caused by a certain concentration of SCFA is dependent on the carbon chain length of the fatty acids. Shorter carbon chains induce increased inhibition of thiamine uptake (Iwashima and Nose 1975).

Nitrogen

The effect of available thiamine concentration on the observed fermentation rate and yeast growth of *S. cerevisiae* has proven to be dependent on the concentration of yeast assimilable nitrogen (YAN) present in must (Bataillon et al. 1996). The

effect of thiamine concentration on fermentation rate and yeast growth was shown to be pronounced at a high nitrogen concentration (300 mg/L YAN), whereas only a marginal change in fermentation rate was effectuated by different thiamine concentrations under conditions with a low nitrogen concentration (60 mg/L YAN). Another study demonstrated that this nitrogen dependent effect is mitigated when thiamine is present in must at saturating levels for yeast growth, which in the case of this study equaled 0.2 mg/L thiamine (Xing 2007).

Other B complex vitamins

The presence of other B complex vitamins such as pyridoxine may affect both thiamine metabolism, as well as the impact of thiamine on yeast growth. Early studies postulate a symbiotic relationship between thiamine and pyridoxine as it is suggested that these vitamins assist in the biosynthesis of one another (Leonian and Lilly 1942; Moses and Joslyn 1953). For instance, pyridoxine is a precursor to HMP, which is utilized for thiamine biosynthesis (Tazuya et al. 1995b; Zeidler et al. 2003). Another study demonstrated that thiamine biosynthesis is terminated in correspondence to a decrease in pyridoxine biosynthesis (Kamihara and Nakamura 1984). It has been proposed that either one of the vitamins may serve as a precursor for the synthesis of the other, as in the last-mentioned case of HMP synthesis, or that one vitamin may be able to functionally substitute the other. This was deduced partly by the ability of *S. cerevisiae* to form carboxylase when only one of thiamine or pyridoxine is available in the culture medium (Moses and Joslyn 1953).

This symbiotic relationship between thiamine and pyridoxine would imply that yeast growth may be sustained as long as one of these vitamins is available in sufficient concentrations. Subsequent studies investigating the interaction effects between thiamine and pyridoxine have, however, demonstrated that this is not always the case. Thiamine addition to a medium deficient in pyridoxine may result in extended pyridoxine deficiency and inhibition of yeast growth, rather than promotion of growth. This extended pyridoxine deficiency leads to terminated heme biosynthesis and respiratory deficiency (Haskell and Snell 1970; Nakamura et al. 1981). The addition of 0.02 μg/mL pyridoxine caused an almost immediate restoration of respiration activity and increased heme production which may facilitate normal growth (Nakamura et al. 1980). The effectivity of thiamine may therefore depend on adequate pyridoxine levels for yeasts in grape must.

A link between the biosynthesis of thiamine and biosynthesis of another B complex vitamin, niacin, has been reported. As discussed above, the HET moiety may be produced in a Thi4-mediated reaction which necessitates NAD^+ as a substrate and releases a form of niacin, namely nicotinamide (Chatterjee et al. 2007). Therefore, Thi4 expression, which is regulated by intracellular thiamine concentration, may affect both thiamine and niacin biosynthesis (Li et al. 2010).

Winery treatments

In addition to the factors mentioned above, winery treatments other than the addition of SO_2, such as pressing, settling, skin contact and racking may all affect the concentration of nutrients in the juice. Approximately 75% of thiamine contents of grapes are found in the juice, and 25% in the skin and pulp, therefore vigorous pressing and extended skin contact may increase must thiamine concentration (Ournac and Flanzy, 1958). Hall et al. (1956) reported that juice extraction from grapes allow for 83% of the total grape thiamine constituent to be available in white musts and all of the total grape thiamine constituent to be available for red musts, most likely due to the extended skin and seed contact implemented for red musts. Juice clarification is another commonly employed practice that is known to cause losses of water-soluble nutrients in grape must (Houtman and Du Plessis, 1986).

Additionally, it has been shown that thiamine levels increase with the addition of lees, as virtually all thiamine from the dead yeasts is transferred to the must (Moreno and Peinado 2012). This implies that cells which die during fermentation may aid other cells by supplying them with thiamine. Such a phenomenon may be of significance during sequential inoculation of *S. cerevisiae* to must initially containing a substantial population of non-*Saccharomyces* yeast, which may die early during fermentation, releasing thiamine into the must. The addition of complex yeast nutrients in cellars may also increase available thiamine concentration as many of these nutrients include inactive dry yeasts which contain vitamins (Pozo-Bayón et al. 2009). In addition, some commercial yeast nutrients added before inoculation, contain supplemented thiamine HCl (Jitjaroen et al. 2009). Managing yeast nutrition to supply yeast with sufficient available thiamine in must is critical to ensure successful fermentation, taking into account all the factors highlighted above and summarized in Fig. 5.

Impact of thiamine on fermentation kinetics and cell viability

Thiamine deficiency is regarded as a major factor that may cause impaired growth, cell death and eventually cessation of fermentations (Alexandre and Charpentier 1998; Bisson 1999; Ough et al. 1989; Ribéreau-Gayon et al. 2006). Indeed, elimination of thiamine from grape must decreases yeast population growth and fermentation rate (Bataillon et al. 1996). On the other hand, the addition of thiamine in a thiamine-deficient must has been shown to restore growth and fermentation rate to the levels observed in the same grape must containing sufficient levels of thiamine (Ough et al. 1989). Bataillon et al. (1996) reported that the addition of 0.25 mg/L thiamine before

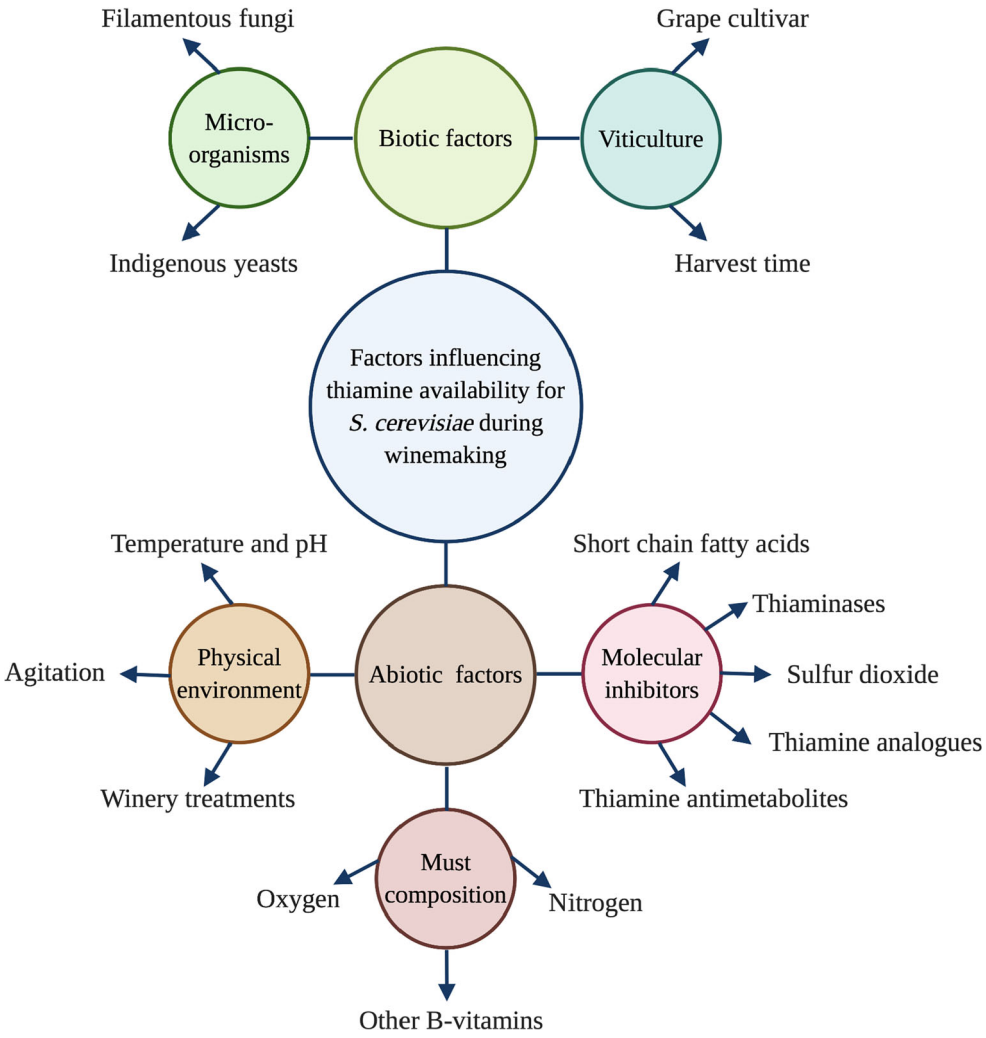

Fig. 5 Schematic summary of factors influencing thiamine availability for *S. cerevisiae* during winemaking. Created with BioRender.com

inoculation ensured adequate fermentation kinetics to complete sugar utilization during oenological fermentation. Moreover, the addition of 0.5 mg/L thiamine to grape must has been reported to increase viable yeast population by up to 30% (Ribéreau-Gayon et al. 2006). Notably, the addition of 0.1 mg/L thiamine to fermentations, which already contained 0.24 g/L thiamine, has shown not to increase sugar utilization or cell growth. This is indicative of a saturation threshold for the effect of thiamine concentration on sugar utilization and fermentation rate (Hucker et al. 2016). From previous literature, it is clear that the absence of exogenous thiamine impairs yeast growth and fermentation kinetics, but it is unclear what is the optimal concentration of thiamine is to maximize these parameters under oenological conditions and to which degree this is strain-dependent and/or subject to environmental conditions. As mentioned in the previous section of this review, most yeast species, including *S. cerevisiae*, are able to synthesize thiamine de novo in grape must deficient of thiamine, but this may only facilitate a very slow fermentation rate and limited cell growth, eventually leading to cessation of fermentations. A low thiamine concentration of 0.025 mg/L affects the length of the yeast growth lag phase in a strain-dependent fashion. This effect is amplified at lower temperatures, most likely as a result of slower metabolism, which may reduce ATP production necessary for thiamine uptake (Ferreira et al. 2017).

The beneficial effect of early thiamine supplementation for yeast during wine fermentations may be attributed to one or a combination of the following: an increase in metabolic enzyme activity as a result of the increased TPP to serve as cofactors, or increased resistance to thermal, osmotic and oxidative stresses as a result of thiamine-dependent protection against stress (Bunik et al. 2013; Wolak et al. 2014). The cofactor function of TPP facilitates the activity of enzymes such as Pdh and Pdc, which are intrinsically involved in glucose utilization via glycolysis and acetaldehyde production via alcoholic fermentation. Therefore, an increase in these enzyme activities, mediated by increased TPP cofactors, may result in increased fermentation rates. Additionally, thiamine is involved in the maintenance of intracellular redox

balance via some TPP-dependent enzymes such as Tkl and Kdh. These enzymes may increase production of NAPDH and glutathione through flux into the PPP during the cells oxidative stress response. Yeasts exhibit oxidative stress under oenological conditions when fermentation conditions are mostly anaerobic. The prevention of this oxidative stress may increase the yeast cell viability (Bunik 2003; Landolfo et al. 2008). In accordance with this oxidative stress response, an up to eight-fold activation of Tkl enzymes has been observed under oxidative stress (Kowalska et al. 2012). Increased activity of enzymes such as Tkl may also aid in increased lipid production to sustain cell viability in conditions of high ethanol during fermentation (Dobrowolski and Mirończuk 2020) as well as increased RNA and DNA production to sustain cell growth. Elevated Kdh activity may also facilitate increased ATP production to sustain a greater fermentation rate and cell growth. As mentioned previously, thiamine may also play a more direct role in exerting protection against stress factors, which may also occur during fermentation. This includes an antioxidative function of thiamine and its derivatives as a result of their ability to interact with and reduce free radicals (Lukienko et al. 2000).

In addition to the positive effects that thiamine availability exhibits with regard to yeast population growth and fermentation rate, it may also alter the production of certain compounds during fermentation. Increased thiamine concentrations in grape must may reduce the synthesis of carbonyl compounds during fermentation, of which significant levels may be produced by yeast. These compounds are known to bind to free sulphur dioxide (SO_2) (Tuite and Oliver 1991). Indeed, addition of 0.5 mg/L thiamine has shown to facilitate decarboxylation of ketone acids such as pyruvic acid and α-ketoglutaric acid, effectively decreasing the accumulation of ketonic compounds that may bind to SO_2 (Lafon-Lafourcade 1983). This ensures increased availability of free SO_2 which can assist in controlling spoilage organisms and oxidation of grape must (Ribéreau-Gayon et al. 2006).

Thiamine availability may ultimately impact wine's sensory properties

As reviewed above, adequate concentration of thiamine in grape must is critical for maximal activity of TPP-dependent enzymes, which in turn may enhance the ability of yeasts to proliferate and complete fermentation at a fast rate. TPP-dependent enzymes are, however, also involved in the formation of numerous compounds that may affect wine aroma. The α-keto acid decarboxylases (Kdc) are important TPP-dependent enzymes in the Ehrlich pathway, catalysing the enzymatic breakdown of amino acid–derived α-keto acids to aldehydes. In turn, these aldehydes can be reduced or oxidized to fusel alcohols or fusel acids respectively (Hazelwood et al. 2008; Dickinson et al. 2003), as illustrated in Fig. 6. These

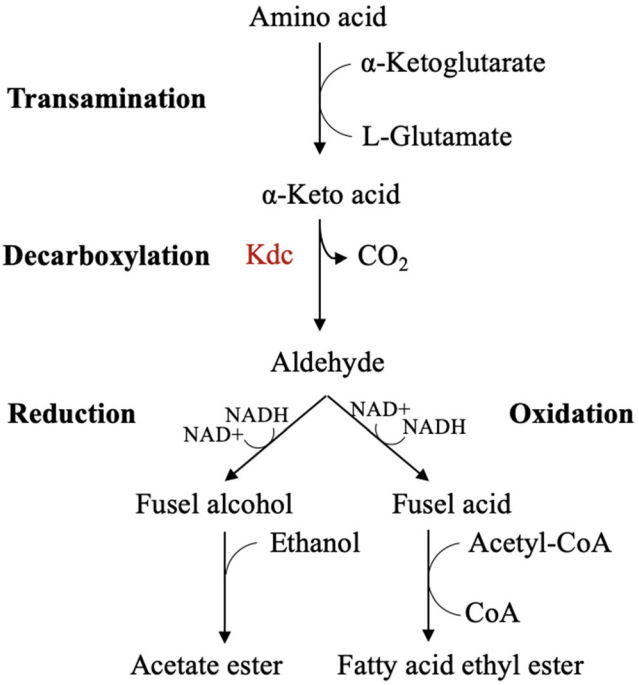

Fig. 6 Aroma formation via amino acid catabolism (Neubauer and Fromherz 1911). Thiamine-dependent enzyme; Kdc, keto acid decarboxylase, indicated in red; CoA, coenzyme A. Redox reactions involving the oxidized and reduced forms of nicotinamide adenine dinucleotide (NAD^+/NADH) are displayed

fusel alcohols can then react with carboxylic acids, to produce acetate esters. Similarly, fusel acids can react with acetyl-CoA to form fatty acid ethyl esters (Bakker and Clarke 2011; Saerens et al. 2010). These fusel alcohols, fusel acids and their respective esters are major contributors to wine aroma (Lambrechts and Pretorius 2000), with both families of esters imparting the most pleasant aromas. The availability of thiamine may impact the activity of these α-keto acid decarboxylases and subsequently alter the aldehyde concentration produced. If fewer aldehydes are produced there will be less substrate available for the next reaction in the pathway.

As aldehydes may be oxidized or reduced, the cellular redox state of the yeast may impact the fate of the α-keto acid. The redox state may be affected by environmental conditions during wine fermentation and when glucose is abundant and conditions are for the most part anaerobic, α-keto acids are reportedly converted primarily to fusel alcohols by *S. cerevisiae* (Boer et al. 2007). It has been hypothesised that the production of fusel alcohols via the reduction step plays a role in maintaining intracellular redox balance during fermentation in an energy efficient manner by oxidation of NADH to NAD^+ (Van dijken and Scheffers 1986). The redox status of the cell has shown to be affected by thiamine availability and the subsequent activity of TPP enzymes (Bunik 2003; Kowalska et al. 2012). Thiamine availability in grape must may therefore affect the yeasts' intracellular redox balance and the corresponding ratio of fusel alcohols to fusel acids

produced during fermentation. Indeed, it has been observed that low thiamine availability reduces the relative ratio of fusel alcohols to fusel acids during fermentation (Jackson 2008).

At least five genes of *S. cerevisiae* have revealed to encode for TPP-dependent decarboxylases that are involved in decarboxylation of α-keto acids within the Ehrlich pathway. These include *PDC1*, *PDC5* and *PDC6* which encode for pyruvate decarboxylase, *THI3* which encodes the decarboxylase involved in leucine catabolism and *ARO10* which encodes other another broad-spectrum decarboxylase (Brion et al. 2014; Dickinson et al. 1997; Ter Schure et al. 1998; Vuralhan et al. 2005). Together, these TPP-dependent enzymes catalyse the decarboxylation of the α-keto acid intermediates of all branched chain amino acids, aromatic amino acids and methionine. As stated before, *THI3* and *PDC2* are known to be involved in the regulation of thiamine uptake and biosynthesis in *S. cerevisiae*, which is dependent on available exogenous thiamine concentration (Nosaka et al. 2005, 2008). The activation of these genes is also involved in regulation of the main pyruvate decarboxylase gene *PDC1* (Brion et al. 2014). *THI3* and *PDC2* may therefore perform a regulatory role in the Ehrlich pathway, which is subject to thiamine availability.

The α-keto acids that may be produced from the catabolism of branched chain amino acids (leucine, isoleucine and valine), aromatic amino acids (tyrosine, tryptophan and phenylalanine), threonine and serine may also originate from central carbon metabolism. Some of these keto acids originate from reactions using pyruvate as precursor. Pyruvate may condense with another pyruvate molecule via a carboligation reaction mediated by the TTP-dependent enzyme, Als. The acetaldehyde formed after this Als-mediated ligation, α-acetolactate, may serve as the intermediate for the production of α-ketoisovalerate and α-ketoisocaproate and subsequent synthesis of respective fusel alcohols and fusel acids. α-Acetolactate may also serve as an intermediate for the formation of aromatic compounds acetoin, 2,3-butanediol and diacetyl. The role of thiamine as a coenzyme for Pdh, which catalyses the conversion of acetaldehyde to acetyl-CoA, also suggests that thiols can react to form aroma-active thiol esters (Kageyama and Murata 2005). In addition, acetyl-CoA and its derivative acyl-CoA may condense with higher alcohols and ethanol respectively to form acetate esters and ethyl esters (Saerens et al. 2010; Selvaraju et al. 2016). Other keto acids from central carbon metabolism, excluding those mentioned from pyruvate catabolism, originate from the shikimate pathway. This pathway first requires flux into the pentose phosphate pathway, which is also facilitated by a TTP-dependent enzyme, Tkl. Thiamine availability could therefore affect the aroma production of yeast during fermentation of carbon sources such as glucose and fructose. The potential impact of TPP-dependent enzyme activity on wine aroma is summarized in Table 2. It should nevertheless be noted that the studies mentioned above have not been performed under winemaking conditions and further experimental work is required to confirm the actual impact on wine aroma.

A deficiency in available thiamine is known to cause inhibited growth and fermentation of yeast, which is not only undesirable for obtaining fast and complete fermentations but may also contribute to synthesis of unwanted compounds for wine aroma. This may result from accumulation of carbonyl compounds, α-keto acids such as pyruvate and α-ketoglutarate, as well as acetaldehyde and phosphoenolpyruvate, which may react to form off-flavours. Increasing the thiamine concentration in must has shown to successfully decrease keto acid concentrations via TPP-dependent decarboxylation, preventing synthesis of these off-flavours (Lafon-

Table 2 Thiamine pyrophosphate–dependent enzymes and their potential impact on wine aroma (Bunik et al. 2013; Demir et al. 2007; Hohmann and Meacock 1998; Kneen et al. 2011; Müller 2014; Valera et al. 2020)

Enzyme	Substrate → product	Impact on the wine aroma
Pyruvate dehydrogenase (Pdh)	Pyruvate → acetyl-CoA	Precursor to thioacetates, acetate esters and ethyl esters
Pyruvate decarboxylase (Pdc)	Various keto acids → aldehydes	Aldehydes and their catabolites from aromatic amino acids, as well as threonine, serine and methionine
α-Ketoglutarate dehydrogenase (Kdh)	α-Ketoglutarate → succinyl-CoA	SO_2 consumption
Transketolase (Tkl)	Fructose-6 phosphate → xylose-5 phosphate	Aldehydes and their catabolites derived from aromatic amino acids, synthesized via the shikimate pathway
Acetolactate synthase (Als)	Pyruvate + pyruvate → α-acetolactate	Acetoin, 2,3-butanediol, diacetyl, compounds derived from valine and leucine
Branched chain α-keto acid dehydrogenase (Bckdh)	α-keto acids of branched chain amino acids → aldehydes	Aldehydes and their catabolites from branched chain amino acids
Phenyl pyruvate decarboxylase	Phenylalanine → 2-phenylethanol	2-phenylethanol
Indole pyruvate decarboxylase	Indole pyruvate → indole acetaldehyde	Indolyl acetic acid
Benzoylformate decarboxylase	Phenylperuvic acid → phenylacetaldehyde	Mandelic acid
Benzaldehyde-lyase	Benzaldehyde + acetaldehyde → acetoin	Acetoin

Lafourcade 1983; Ribéreau-Gayon et al. 2006). Reduction of keto acids in must also prevents the reaction and binding of these compounds to bisulfite, which is present in must after SO_2 addition (Tuite and Oliver 1991). The binding of bisulfite to keto acids and free acetaldehyde molecules not only makes it impossible for yeast to assimilate and metabolize these precursors to aroma-active compounds during the full duration of fermentation (Ochando et al. 2020) but SO_2 may be released from this bound complex at a later stage during wine ageing, which may be perceived as a sharp unpleasant odour above a sensory threshold of 100 mg/L (Blesic et al. 2014).

In addition to accumulation of carbonyl compounds, a lack of TPP may affect the formation of hydrogen sulfide (H_2S), an undesirable compound which may impart off-flavours to wine (Franco-Luesma et al. 2016; Mestres et al. 2000). H_2S is ordinarily formed during oenological fermentation by the reduction of exogenous sulfate (Schütz and Kunkee 1977). Sufficient thiamine concentration has shown to cause significantly less H_2S to be present in a synthetic grape juice medium. This could be ascribed to the fact that under thiamine-limited conditions, synthesis of acetyl-CoA is reduced, which in turn reduces sulphur metabolism via amino acid synthesis, allowing greater sulphide concentrations to be liberated as H_2S (Thomas and Surdin-Kerjan 1997; Xing 2007). Additionally, it has been demonstrated that increased H_2S production is correlated with an increased expression of genes involved in thiamine biosynthesis genes (Bartra et al. 2010). Therefore, an abundance of exogenous thiamine which inactivates expression of these genes (Nosaka et al. 2005) decreases H_2S formation.

Conclusions and future outlooks

Nutrient availability is an essential factor that influences the ability of yeasts to grow. Our understanding of yeast requirements for specific nutrients may determine the successful utilization of yeasts for various commercial and industrial applications. Thiamine is one of the essential nutrients for metabolic processes involved in growth and proliferation of yeast, including carbon assimilation and the production of DNA, RNA, several lipids, antioxidants and amino acids. Additionally, thiamine may also serve an important role in protection of yeast against external stress factors. From an oenological point of view, it is well documented that yeasts such as *Saccharomyces cerevisiae* may benefit from increased thiamine availability, which significantly increases growth and fermentation rates. This review reiterates that the levels of thiamine produced by prototrophic wine yeast are not adequate for maximum fermentation benefits. Considering that yeasts are able to synthesize as well as assimilate exogenous thiamine, two possible routes for mitigation of this issue exist in: optimized de novo synthesis, as well as optimized thiamine assimilation in addition to ensuring sufficient assimilable exogenous thiamine.

Increased de novo synthesis of thiamine by *S. cerevisiae* would reduce expenses involved with thiamine supplementation; however, seeing as yeasts are adapted to rapidly assimilate great amounts of exogenous thiamine early during fermentation, it is often opted only to ensure that exogenous thiamine concentration is sufficient. Many factors affect the availability of exogenous thiamine in must including the grape variety used and subsequent likely depletion of thiamine by indigenous microorganisms (Bataillon et al. 1996). Several abiotic physiochemical factors including must composition, yeast-produced factors and winemaking practices may affect the molecular stability of thiamine in must or alter thiamine uptake and utilization thereof by yeasts after inoculation. Many of the effects of these factors on thiamine availability, uptake and synthesis are not yet fully understood or thoroughly researched. In particular, little is known about the effect of various viticultural and winemaking practices on thiamine concentration of grapes, at which levels thiaminases are present in grape must, and what impact oxygen availability and agitation have on thiamine uptake and synthesis. Multifactorial studies investigating the impact of these factors and their interactions on thiamine uptake and assimilation may aid in a better understanding of yeast thiamine metabolism under oenological conditions.

One of the limitations for these studies includes the large number of factors and their interactions which may affect yeast thiamine metabolism. This is in part due to the complexity of the grape must matrix, which itself poses the limitation of obtaining accurate measurements of the nutrient content of must during fermentation. At present, thiamine concentration may be quantified using several techniques including fluorescence, colorimetric, electrochemical and biological methods, but the development of faster, more accurate and more accessible methods may aid research on yeast nutrition. Accurate quantification of exogenous as well as intracellular thiamine concentrations under oenological conditions is paramount for future research investigating thiamine metabolism during winemaking and will provide useful information for optimising thiamine levels in must. At a more fundamental level, an enhanced understanding of genetic regulation and biochemistry of thiamine metabolism would aid in attempts to increase thiamine metabolism of yeast. This review, for instance, highlights that an alternative pathway involved in the anaerobic biosynthesis of HMP, a precursor to thiamine, remains to be determined. In addition, evidence implicating a role of NAD^+ metabolism and redox balance in thiamine gene regulation adds to our incomplete understanding of thiamine gene regulation.

This review focuses mainly on thiamine metabolism of *S. cerevisiae*, on account of the limited research regarding thiamine metabolism for other yeast species. It is known that

some yeast species lack key genes required for thiamine biosynthesis and some species possess more copies of these genes than found in *S. cerevisiae* (Perli et al. 2020). Future studies may therefore investigate potential differences in thiamine metabolism of different yeast species. This may be of particular interest for industrial applications such as winemaking, as grape must contains various indigenous yeast, and co- or sequential inoculation of *S. cerevisiae* with other yeast species is a familiar practice. An enhanced understanding of yeast thiamine requirements may allow for superior management of yeast nutrition to ensure optimal fermentation results.

Authors' contributions PWJL and BD conceived the review. PWJL wrote the manuscript. BD edited it. Both authors approved the final version.

Funding This work is based on the research supported in part by the National Research Foundation of South Africa (Grant Number: 113303), the Faculty of AgriSciences' bursary scheme at Stellenbosch University and Lallemand SAS.

Compliance with ethical standards

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest The authors declare that they have no conflict of interest.

References

Aceituno FF, Orellana M, Torres J, Mendoza S, Slater AW, Melo F, Agosin E (2012) Oxygen response of the wine yeast *Saccharomyces cerevisiae* EC1118 grown under carbon-sufficient, nitrogen-limited enological conditions. Appl Environ Microbiol 78: 8340–8352

Alexandre H, Charpentier C (1998) Biochemical aspects of stuck and sluggish fermentation in grape must. J Ind Microbiol Biotechnol 20:20–27

Amata I (2013) Yeast a single cell protein: characteristics and metabolism. Int J Appl Biol Pharm 4:158–170

Bakker J, Clarke RJ (2011) Wine: flavour chemistry. John Wiley & Sons, Hoboken

Bardi L, Cocito C, Marzona M (1999) *Saccharomyces cerevisiae* cell fatty acid composition and release during fermentation without aeration and in absence of exogenous lipids. Int J Food Microbiol 47: 133–140

Bartra E, Casado M, Carro D, Campamà C, Piña B (2010) Differential expression of thiamine biosynthetic genes in yeast strains with high and low production of hydrogen sulfide during wine fermentation. J Appl Microbiol 109:272–281

Bataillon M, Rico A, Sablayrolles JM, Salmon JM, Barre P (1996) Early thiamin assimilation by yeasts under enological conditions: impact on alcoholic fermentation kinetics. J Ferment Bioeng 82:145–150

Begley TP (1996) The biosynthesis and degradation of thiamin (vitamin B1). Nat Prod Rep 13:177–185

Bhagavan NV (2002) Vitamin metabolism. In: Medical biochemistry, 4th edn. Academic press, Cambridge, pp 901–928

Bisson LF (1999) Stuck and sluggish fermentations. Am J Enol Vitic 50: 107–119

Blesic M, Mujakic V, Spaho N, Smajic-Murtic M (2014) Threshold of sensory perception of sulphur dioxide in Herzegovinian white wines [Conference poster]. In: 25th Scientific-Experts Congress on Agriculture and Food Industry, Izmir, 25-27 September 2014. Poster Session, 89-92

Boer VM, Tai SL, Vuralhan Z, Arifin Y, Walsh MC, Piper MDW, de Winde JH, Pronk JT, Daran J-M (2007) Transcriptional responses of *Saccharomyces cerevisiae* to preferred and nonpreferred nitrogen sources in glucose-limited chemostat cultures. FEMS Yeast Res 7: 604–620

Boulton RB, Singleton VL, Bisson LF, Kunkee RE (1996) Principles and practices of winemaking. Chapman Hall, New York

Brion C, Ambroset C, Delobel P, Sanchez I, Blondin B (2014) Deciphering regulatory variation of *THI* genes in alcoholic fermentation indicate an impact of Thi3p on *PDC1* expression. BMC Genom 15:1085

Broach JR (2012) Nutritional control of growth and development in yeast. Genet 192:73–105

Bunik VI (2003) 2-Oxo acid dehydrogenase complexes in redox regulation. Eur J Biochem 270:1036–1042

Bunik VI, Tylicki A, Lukashev NV (2013) Thiamin diphosphate-dependent enzymes: from enzymology to metabolic regulation, drug design and disease models. FEBS J 280:6412–6442

Burrows RJ, Byrne KL, Meacock PA (2000) Isolation and characterization of *Saccharomyces cerevisiae* mutants with derepressed thiamine gene expression. Yeast 16:1497–1508

Bussey H, Umbarger HE (1969) Biosynthesis of branched-chain amino acids in yeast: regulation of synthesis of the enzymes of isoleucine and valine biosynthesis. J Bacteriol 98:623–628

Cambon B, Monteil V, Remize F, Camarasa C, Dequin S (2006) Effects of *GPD1* overexpression in *Saccharomyces cerevisiae* commercial wine yeast strains lacking *ALD6* genes. Appl Environ Microbiol 72: 4688–4694

Campobasso N, Costello CA, Kinsland C, Begley TP, Ealick SE (1998) Crystal structure of thiaminase-I from *Bacillus thiaminolyticus* at 2.0 A resolution. Biochemistry 37:15981–15989

Chatterjee A, Jurgenson CT, Schroeder FC, Ealick SE, Begley TP (2007) Biosynthesis of thiamin thiazole in eukaryotes: conversion of NAD to an advanced intermediate. J Am Chem Soc 14:2914–2922

Chatterjee A, Abeydeera ND, Bale S, Pai PJ, Dorrestein PC, Russell DH, Ealick SE, Begley TP (2011) *Saccharomyces cerevisiae* Thi4p is a suicide thiamine thiazole synthase. Nature 478:542–546

Cressina E, Chen L, Moulin M, Leeper FJ, Abell C, Smith AG (2011) Identification of novel ligands for thiamine pyrophosphate (TPP) riboswitches. Biochem Soc Trans 39:652–657

Daudt CE, Parizzi LE (1995) Tiamina e riboflavina: evolução com a maturação de Cabernet Sauvignon e comportamento durante a fermentação com diferentes níveis de anidrido sulfuroso adicionado. Ciência Rural 25:311–314

Demir AS, Ayhan P, Sopaci SB (2007) Thiamine pyrophosphate dependent enzyme catalyzed reactions: stereoselective C–C bond formations in water. CLEAN – Soil, Air, Water 35:406–412

Dickinson JR, Dawes IW (1992) The catabolism of branched-chain amino acids occurs via 2-oxoacid dehydrogenase in *Saccharomyces cerevisiae*. J Gen Microbiol 138:2029–2033

Dickinson JR, Lanterman MM, Danner DJ, Pearson BM, Sanz P, Harrison SJ, Hewlins MJ (1997) A ^{13}C nuclear magnetic resonance investigation of the metabolism of leucine to isoamyl alcohol in *Saccharomyces cerevisiae*. J Biol Chem 272:26871–26878

Dickinson JR, Salgado LEJ, Hewlins MJ (2003) The catabolism of amino acids to long chain and complex alcohols in *Saccharomyces cerevisiae*. J Biol Chem 278:8028–8034

Dittrich HH, Sponholz WR (1975) Die aminosaureabnahme in *Botrytis*-infizierten Traubenbeeren und die Bilund hoherer Alkohole in deisen Mosten bei ihrer Vergarung. Wein-Wissen 30:188–210

Dobrowolski A, Mirończuk AM (2020) The influence of transketolase on lipid biosynthesis in the yeast *Yarrowia lipolytica*. Microbial Cell Fact 19:1–9

Dwivedi BK, Arnold RG (1973) Chemistry of thiamine degradation on food products and model systems. Review. J Agric Food Chem 21: 54–60

Dyda F, Furey W, Swaminathan S, Sax M, Farrenkopf B, Jordan F (1993) Catalytic centers in the thiamin diphosphate dependent enzyme pyruvate decarboxylase at 2.4-.ANG. resolution. Biochemistry 32: 6165–6170

Edwards KA, Tu-Maung N, Cheng K, Wang B, Baeumner AJ, Kraft CE (2017) Thiamine assays—advances, challenges, and caveats. ChemistryOpen 6:178–191

Enjo F, Nosaka K, Ogata M, Iwashima A, Nishimura H (1997) Isolation and characterization of a thiamin transport gene, *THI10*, from *Saccharomyces cerevisiae*. J Biol Chem 272:19165–19170

Ensom MH, Decarie D (2005) Stability of thiamine in extemporaneously compounded suspensions. Can J Hosp Pharm 58:26–30

Estramareix B (1996) Biosynthesis of thiamine. New J Chem 20:607–629

Ferreira D, Galeote V, Sanchez I, Legras JL, Ortiz-Julien A, Dequin S (2017) Yeast multistress resistance and lag-phase characterisation during wine fermentation. FEMS Yeast Res 17:fox051

Franco-Luesma E, Sáenz-Navajas MP, Valentin D, Ballester J, Rodrigues H, Ferreira V (2016) Study of the effect of H_2S, MeSH and DMS on the sensory profile of wine model solutions by Rate-All-That-Apply (RATA). Food Res Int 87:152–160

Haas AL, Laun NP, Begley TP (2005) Thi20, a remarkable enzyme from *Saccharomyces cerevisiae* with dual thiamin biosynthetic and degradation activities. Bioorg Chem 33:338–344

Haddad GS, Loewenstein M (1983) Effect of several heat treatments and frozen storage on thiamine, riboflavin, and ascorbic acid content of milk. J Dairy Sci 66:1601–1606

Hall AP, Brinner L, Amerine MA, Morgan AF (1956) The B vitamin content of grapes, musts and wines. Food Res 21:362–371

Hanes JW, Burns KE, Hilmey DG, Chatterjee A, Dorrestein PC, Begley TP (2008) Mechanistic studies on pyridoxal phosphate synthase: the reaction pathway leading to a chromophoric intermediate. J Am Chem Soc 130:3043–3052

Haskell BE, Snell EE (1970) Microbiological determination of the vitamin B6 group. Meth Enzym 18:512–519

Hazelwood LA, Daran JM, van Maris AJ, Pronk JT, Dickinson JR (2008) The Ehrlich pathway for fusel alcohol production: a century of research on *Saccharomyces cerevisiae* metabolism. App Environ Microbiol 74:2259–2266

Hohmann S, Meacock PA (1998) Thiamin metabolism and thiamin diphosphate-dependent enzymes in the yeast *Saccharomyces cerevisiae*: genetic regulation. Biochim Biophys Acta 1385:201–219

Holz M, Otto C, Kretzschmar A, Yovkova V, Aurich A, Pötter M, Marx A, Barth G (2011) Overexpression of alpha-ketoglutarate dehydrogenase in *Yarrowia lipolytica* and its effect on production of organic acids. Appl Microbiol Biotechnol 89:1519–1526

Houtman AC, Du Plessis CS (1986) Nutritional deficiencies of clarified white grape juices and their correction in relation to fermentation. S Afr J Enol Vitic 7:39–46

Hucker B, Wakeling L, Vriesekoop F (2016) Vitamins in brewing: presence and influence of thiamine and riboflavin on wort fermentation. J Inst Brew 122:126–137

Iwashima A, Nose Y (1975) Inhibition of thiamine transport in anaerobic baker's yeast by iodoacetate, 2,4-dinitrophenol N,N'-dicyclohexylcarbodiimide and fatty acids. Biochim Biophys Acta 399:375–383

Iwashima A, Nose Y (1976) Regulation of thiamine transport in *Saccharomyces cerevisiae*. J Bacteriol 128:855–857

Iwashima A, Nishino H, Nose Y (1973) Carrier-mediated transport of thiamine in baker's yeast. Biochim Biophys Acta 330:222–234

Iwashima A, Wakabayashi Y, Nose Y (1975) Thiamine transport mutants of *Saccharomyces cerevisiae*. Biochim Biophys Acta 413:243–247

Iwashima A, Nishimura H, Nose Y (1979) Soluble and membrane-bound thiamine-binding proteins from *Saccharomyces cerevisiae*. Biochim Biophys Acta 557:460–468

Iwashima A, Nosaka K, Nishimura H, Kimura Y (1986) Some properties of a *Saccharomyces cerevisiae* mutant resistant to 2-amino-4-methyl-5-β-hydroxyethylthiazole. J Gen Microbiol 132:1541–1546

Iwashima A, Kawasaki Y, Kimura Y (1990) Transport overshoot during 2-methyl-4-amino-5-hydroxymethylpyrimidine uptake by *Saccharomyces cerevisiae*. Biochim Biophys Acta 1028:161–164

Jackson RS (2008) Wine science: principles and applications, 3rd edn. Academic press, Cambridge

Jenkins AH, Schyns G, Potot S, Sun G, Begley TP (2007) A new thiamin salvage pathway. Nat Chem Biol 3:492–497

Jitjaroen W, Sponholz WR, Noga G (2009) Relationship between yeast strains and nutritive supplements and its influence on enological parameters of santol (sandoricum koetjape Merr.) wine production. Witthayasan Kasetsat 43:550–556

Joslyn MA (1951) Nutrient requirements of yeast. Mycopathol Mycol Appl 5:260–276

Juhász O, Dworschák E, Kozma P (1987) Nutritive value of different grape musts (*Vitis vinifera* L.). Plant Foods Hum Nutr 37:275–281

Julies JM (2019) Evaluating the vitamin requirements of wine-related yeasts and the resultant impact on population dynamics and fermentation kinetics. MSc dissertation, Stellenbosch University

Kageyama Y, Murata S (2005) Oxidative formation of thiolesters in a model system of the pyruvate dehydrogenase complex. J Org Chem 70:3140–3147

Kamihara T, Nakamura I (1984) Regulation of respiration and its related metabolism by vitamin B 1 and vitamin B 6 in *Saccharomyces* yeasts. In: Fiechter A (ed) Immobilized biocatalysts *Saccharomyces* yeasts wastewater treament. Springer, Berlin, pp 35–82

Kawasaki C, Shinoda S, Ono T (1967) The growth-stimulating activity of thiamine derivatives on thiamine-requiring microorganisms. XV. Accumulation of thiamine derivatives on the cells of *Kloeckera apiculata*. Vitamin 36:535–540

Kneen MM, Stan R, Yep A, Tyler RP, Saehuan C, McLeish MJ (2011) Characterization of a thiamin diphosphate-dependent phenylpyruvate decarboxylase from *Saccharomyces cerevisiae*. FEBS J 278:1842–1853

Kowalska E, Kujda M, Wolak N, Kozik A (2012) Altered expression and activities of enzymes involved in thiamine diphosphate biosynthesis in *Saccharomyces cerevisiae* under oxidative and osmotic stress. FEMS Yeast Res 12:534–546

Lafon-Lafourcade S (1983) Wine and brandy. In: Rehm HJ, Reed G (eds) Food and feed production with microorganisms (biotechnology), 5th edn. Weinheim, Verlag Chemie, pp 81–163

Lakaye B, Wirtzfeld B, Wins P, Grisar T, Bettendorff L (2004) Thiamine triphosphate, a new signal required for optimal growth of *Escherichia coli* during amino acid starvation. J Biol Chem 279: 17142–17147

Lambrechts MG, Pretorius IS (2000) Yeast and its importance to wine aroma-a review. S Afr J Enol Vitic 21:97–129

Landolfo S, Politi H, Angelozzi D, Mannazzu I (2008) ROS accumulation and oxidative damage to cell structures in *Saccharomyces cerevisiae* wine strains during fermentation of high-sugar-containing medium. Biochim Biophys Acta 1780:892–898

Large PJ (1986) Degradation of organic nitrogen compounds by yeasts. Yeast 2:1–34

Leichter J, Joslyn MA (1969) Kinetics of thiamin cleavage by sulphite. Biochem J 113:611–615

Leonian LH, Lilly VG (1942) The effect of vitamins on ten strains of *Saccharomyces cerevisiae*. Am J Bot 29:459–464

Li M, Petteys BJ, McClure JM, Valsakumar V, Bekiranov S, Frank EL, Smith JS (2010) Thiamine biosynthesis in *Saccharomyces cerevisiae* is regulated by the NAD^+-dependent histone deacetylase Hst1. Mol Cell Biol 30:3329–3341

Llorente B, Fairhead C, Dujon B (1999) Genetic redundancy and gene fusion in the genome of the baker's yeast *Saccharomyces cerevisiae*: functional characterization of a three-member gene family involved in the thiamine biosynthetic pathway. Mol Microbiol 32:1140–1152

Lukienko PI, Mel'nichenko NG, Zverinskii IV, Zabrodskaya SV (2000) Antioxidant properties of thiamine. Bull Exp Biol Med 130:874–876

Machado CR, Praekelt UM, Costa de Oliveira RL, Barbosa ACC, Byrne KL, Meacock PA, Menck CFM (1997) Dual role for yeast *THI4* gene in thiamine biosynthesis and DNA damage tolerance. J Mol Biol 273:114–121

Makarchikov AF, Lakaye B, Gulyai IE, Czerniecki J, Coumans B, Wins P, Grisar T, Bettendorff L (2003) Thiamine triphosphate and thiamine triphosphatase activities: from bacteria to mammals. Cell Mol Life Sci 60:1477–1488

Malandrinos G, Louloudi M, Hadjiliadis N (2006) Thiamine models and perspectives on the mechanism of action of thiamine-dependent enzymes. Chem Soc Rev 35:684–692

Marobbio CM, Vozza A, Harding M, Bisaccia F, Palmieri F, Walker JE (2002) Identification and reconstitution of the yeast mitochondrial transporter for thiamine pyrophosphate. EMBO J 21:5653–5661

Mauri LM, Alzamora SM, ChirifeJ TMJ (2007) Review: kinetic parameters for thiamine degradation in foods and model solutions of high water activity. Int J Food Sci 24:1–9

Medina-Silva R, Barros MP, Galhardo RS, Netto LS, Colepicolo P, Menck CF (2005) Heat stress promotes mitochondrial instability and oxidative responses in yeast deficient in thiazole biosynthesis. Res Microbiol 157:275–281

Mestres M, Busto O, Guasch J (2000) Analysis of organic sulfur compounds in wine aroma. J Chromatogr A 881:569–581

Mojzita D, Hohmann S (2006) Pdc2 coordinates expression of the *THI* regulon in the yeast *Saccharomyces cerevisiae*. Mol Genet Genomics 276:147–161

Moreno J, Peinado R (2012) Enological chemistry. Academic Press, Cambridge

Moses W, Joslyn MA (1953) The equivalence of thiamin and pyridoxine for a strain of *Saccharomyces cerevisiae* I: effect on growth rate and carboxylase activity. J Bacteriol 66:197–203

Müller N (2014) Thiamine diphosphate - a natural iminium salt. Its role in microbiological processes during wine preparation and on the aroma of wine. Z Naturforsch 69b:489–500

Muller EH, Richards EJ, Norbeck J, Byrne KL, Karlsson K, Pretorius GHJ, Meacock P, Blomberg A, Hohmann S (1999) Thiamine repression and pyruvate decarboxylase autoregulation independently control the expression of the *Saccharomyces cerevisiae PDC5* gene. FEBS Lett 449:245–250

Nakamura I, Isobe N, Kamihara T, Fukui S (1980) Effects of thiamine and pyridoxine on respiratory activity in *Saccharomyces carlsbergensis* strain 4228. Arch Microbiol 127:47–51

Nakamura I, Isobe N, Nakamura N, Kamihara T, Fukui S (1981) Mechanism of thiamine-induced respiratory deficiency in *Saccharomyces carlsbergensis*. J Bacteriol 147:954–961

Nakamura I, Ohmura Y, Nagami Y, Kamihara T, Fukui S (1982) Thiamin accumulation and growth inhibition in yeasts. Microbiology 128: 2601–2609

Neal AL, Weinstock JO, Lampen JO (1965) Mechanisms of fatty acid toxicity for yeast. J Bacteriol 90:126–131

Neubauer O, Fromherz K (1911) Über den Abbau der Aminosaüren bei der Hefegärung. Hoppe-Seylers Z Physiol Chem 70:326–350

Nielsen J (2014) Synthetic biology for engineering acetyl coenzyme A metabolism in yeast. mBio 5:e02153–e02114

Nishimura H, Kawasaki Y, Kaneko Y, Nosaka K, Iwashima A (1992a) A positive regulatory gene, *THI3*, is required for thiamine metabolism in *Saccharomyces cerevisiae*. J Bacteriol 174:4701–4706

Nishimura H, Kawasaki Y, Kaneko Y, Nosaka K, Iwashima A (1992b) Cloning and characteristics of a positive regulatory gene, *THI2*(*PHO6*), of thiamin biosynthesis in *Saccharomyces cerevisiae*. FEBS Lett 297:155–158

Nosaka K (2006) Recent progress in understanding thiamin biosynthesis and its genetic regulation in *Saccharomyces cerevisiae*. Appl Microbiol Biotechnol 72:30–40

Nosaka K, Kaneko Y, Nishimura H, Iwashima A (1989) A possible role for acid phosphatase with thiamin-binding activity encoded by *PHO3* in yeast. FEMS Microbiol Lett 60:55–59

Nosaka K, Kaneko Y, Nishimura H, Iwashima A (1993) Isolation and characterization of a thiamin pyrophosphokinase gene, *THI80*, from *Saccharomyces cerevisiae*. J Biol Chem 268:17440–17447

Nosaka K, Nishimura H, Kawasaki Y, Tsujihara T, Iwashima A (1994) Isolation and characterization of the *THI6* gene encoding a bifunctional thiamin-phosphate pyrophosphorylase/hydroxyethylthiazole kinase from *Saccharomyces cerevisiae*. J Biol Chem 269:30510–30516

Nosaka K, Onozuka M, Konno H, Kawasaki Y, Nishimura H, Sano M, Akaji K (2005) Genetic regulation mediated by thiamin pyrophosphate-binding motif in *Saccharomyces cerevisiae*. Mol Microbiol 58:467–479

Nosaka K, Onozuka M, Konno H, Akaji K (2008) Thiamin-dependent transactivation activity of *PDC2* in *Saccharomyces cerevisiae*. FEBS Lett 582:3991–3996

Ochando T, Mouret JR, Humbert-Goffard A, Aguera E, Sablayrolles JM, Farines V (2020) Comprehensive study of the dynamic interaction between SO_2 and acetaldehyde during alcoholic fermentation. Food Res Int 136:109607

OIV (2020) International code of oenological practices. International Organisation of Vine and Wine, Paris

Okonji R, Agboola O (2014) Comparative study of some enzymes in different varieties of fruits. Int J Biol Chem Sci 8:419–425

Onozuka M, Konno H, Kawasaki Y, Akaji K, Nosaka K (2008) Involvement of thiaminase II encoded by the *THI20* gene in thiamin salvage of *Saccharomyces cerevisiae*. FEMS Yeast Res 8:266–275

Ough CS, Davenport M, Joseph K (1989) Effects of certain vitamins on growth and fermentation rate of several commercial active dry wine yeasts. Am J Enol Vitic 40:208–213

Ournac A, Flanzy M (1958) Localisation et évolution de la vitamine B1 dans le raisin au cours de la maturation. Qual Plant Mater Veg 3-4: 415–420

Panijpan B, Ratanaubolchai K (1980) Kinetics of thiamine-polyphenol interactions and mechanism of thiamine disulphide formation. Int J Vitam Nutr Res 50:247–253

Patel MS, Nemeria NS, Furey W, Jordan F (2014) The pyruvate dehydrogenase complexes: structure-based function and regulation. J Biol Chem 289:16615–16623

Perli T, Wronska AK, Ortiz-Merino RA, Pronk JT, Daran JM (2020) Vitamin requirements and biosynthesis in *Saccharomyces cerevisiae*. Yeast 37:283–304

Peynaud E, Lafourcade S (1958) Évolution des vitamines B dans le raisin. Qual Plant Mat Veg 3-4:405–414

Pohl M, Sprenger GA, Müller M (2004) A new perspective on thiamine catalysis. Curr Opin Biotechnol 15:335–342

Pozo-Bayón MÁ, Andújar-Ortiz I, Moreno-Arribas MV (2009) Scientific evidences beyond the application of inactive dry yeast preparations in winemaking. Food Res Int 42:754–761

Praekelt UM, Byrne KL, Meacock PA (1994) Regulation of *THI4* (*MOL1*), a thiamine-biosynthetic gene of *Saccharomyces cerevisiae*. Yeast 10:481–490

Raghuram V, Lobo Z, Maitra PK (1994) *PDC2*, a yeast gene essential for synthesis of pyruvate decarboxylase, encodes a novel transcription factor. J Genet 73:17–32

Rapala-Kozik M, Kowalska E, Ostrowska K (2008) Modulation of thiamine metabolism in Zea mays seedlings under conditions of abiotic stress. J Exp Bot 59:4133–4143

Remize F, Andrieu E, Dequin S (2000) Engineering of the pyruvate dehydrogenase bypass in *Saccharomyces cerevisiae*: role of the cytosolic Mg^{2+} and mitochondrial K^{+} acetaldehyde dehydrogenases Ald6p and Ald4p in acetate formation during alcoholic fermentation. Appl Environ Microbiol 66:3151–3159

Ribéreau-Gayon J, Peynaud E, Ribéreau-Gayon P, Sudraud P (1975) Traité d'oenologie. Sciences et techniques du vin. Tome 2. Dunod, Paris

Ribéreau-Gayon P, Glories Y, Maujean A, Dubourdieu D (2006) Handbook of enology, volume 2: the chemistry of wine-stabilization and treatments. John Wiley & Sons, Hoboken

Rintala E, Toivari M, Pitkänen JP, Wiebe MG, Ruohonen L, Penttilä M (2009) Low oxygen levels as a trigger for enhancement of respiratory metabolism in *Saccharomyces cerevisiae*. BMC Genomics 10: 461

Rodríguez-Navarro S, Llorente B, Rodriguez-Manzaneque MT, Ramne A, Uber G, Marchesan D, Dujon B, Herrero E, Sunnerhagen P, Perez-Ortin JE (2002) Functional analysis of yeast gene families involved in metabolism of vitamins B1 and B6. Yeast 19:1261–1276

Rollero S, Bloem A, Ortiz-Julien A, Camarasa C, Divol B (2018) Altered fermentation performances, growth, and metabolic footprints reveal competition for nutrients between yeast species inoculated in synthetic grape juice-like medium. Front Microbiol 9:196

Rosenfeld E, Beauvoit B, Blondin B, Salmon JM (2003) Oxygen consumption by anaerobic *Saccharomyces cerevisiae* under enological conditions: effect on fermentation kinetics. Appl Environ Microbiol 69:113–121

Saerens SMG, Delvaux FR, VerstrepenKJ TJM (2010) Production and biological function of volatile esters in *Saccharomyces cerevisiae*. Microbial Biotechnol 3:165–177

Samson FE, Katz AM, Harris DL (1955) Effects of acetate and other short-chain fatty acids on yeast metabolism. Arch Biochem Biophys 54:406–423

Savocco J, Nootens S, Afokpa W, Bausart M, Chen X, Villers J, Renard HF, Prévost M, Wattiez R, Morsomme P (2019) Yeast α-arrestin Art2 is the key regulator of ubiquitylation-dependent endocytosis of plasma membrane vitamin B1 transporters. PLoS Biol 17:e3000512

Schellenberger A, Hübner G, Neef H (1997) Cofactor designing in functional analysis of thiamin diphosphate enzymes. Meth Enzym 279: 131–146

Schenk G, Duggleby RG, Nixon PF (1998) Properties and functions of the thiamin diphosphate dependent enzyme transketolase. Int J Biochem Cell Biol 30:1297–1318

Schütz M, Gafner J (1993) Analysis of yeast diversity during spontaneous and induced alcoholic fermentations. J Appl Bacteriol 75:551–558

Schütz M, Kunkee RE (1977) Formation of hydrogen sulfide from elemental sulfur during fermentation by wine yeast. Am J Enol Vitic 28:137–144

Selvaraju K, Gowsalya R, Vijayakumar R, Nachiappan V (2016) MGL2/YMR210w encodes a monoacylglycerol lipase in *Saccharomyces cerevisiae*. FEBS Lett 590:1174–1186

Shimazono N, Katsura E (1965) Review of Japanese literature on beriberi and thiamine. Vitamin B Research Committee of Japan. Igaku Shoin, Tokyo

Shimizu M, Masuo S, Itoh E, Zhou S, Kato M, Takaya N (2016) Thiamine synthesis regulates the fermentation mechanisms in the fungus *Aspergillus nidulans*. Biosci Biotechnol Biochem 80:1768–1775

Singleton CK (1997) Identification and characterization of the thiamine transporter gene of *Saccharomyces cerevisiae*. Gene 199:111–121

Stieglitz B, Levy R, Mateles TI (1974) Thiamine accumulation in yeast. J Appl Chem Biotechnol 24:277–282

Stincone A, Prigione A, Cramer T, Wamelink MM, Campbell K, Cheung E, Olin-Sandoval V, Grüning NM, Krüger A, Tauqeer Alam M, Keller MA, Breitenbach M, Brindle KM, Rabinowitz JD, Ralser M (2015) The return of metabolism: biochemistry and physiology of the pentose phosphate pathway. Biol Rev Camb Philos Soc 90: 927–963

Suomalainen H, Oura E (1971) In: Rose AH, Harrison SJ (eds) The yeasts, vol 2. Academic Press, New York, p 374

Tanaka K, Tazuya K, Yamada K, Kumaoka H (2000) Biosynthesis of thiamin under anaerobic conditions in *Saccharomyces cerevisiae*. Biol Pharm Bull 23:108–111

Tanphaichitr V (1999) Thiamin. In: Shils ME, Olsen JA, Shike M (eds) Modern nutrition in health and disease, 9th edn. Lippincott Williams & Wilkins, Baltimore, pp 381–389

Tazuya K, Adachi Y, Masuda K, Yamada K, Kumaoka H (1995a) Origin of the nitrogen atom of pyridoxine in *Saccharomyces cerevisiae*. Biochim Biophys Acta 1244:113–116

Tazuya K, Azumi C, Yamada K, Kumaoka H (1995b) Pyrimidine moiety of thiamin is biosynthesized from pyridoxine and histidine in *Saccharomyces cerevisiae*. Biochem Mol Biol Int 36:883–888

Ter Schure EG, Flikweert MT, van Dijken JP, Pronk JT, Verrips CT (1998) Pyruvate decarboxylase catalyzes decarboxylation of branched-chain 2-oxo acids but is not essential for fusel alcohol production by *Saccharomyces cerevisiae*. Appl Environ Microbiol 64:1303–1307

Thomas D, Surdin-Kerjan Y (1997) Metabolism of sulfur amino acids in *Saccharomyces cerevisiae*. Microbiol Mol Biol Rev 61:503–532

Trevelyan WE, Harrison JS (1954) Studies on yeast metabolism. IV. The effect of thiamine on yeast fermentation. Biochem J 57:561–566

Tuite MF, Oliver SG (1991) Biochemical techniques. In: *Saccharomyces*. Springer, Boston, pp 283–320

Tylicki A, Czerniecki J, Dobrzyn P, Matanowska A, Olechno A, Strumilo S (2005) Modification of thiamine pyrophosphate dependent enzyme activity by oxythiamine in *Saccharomyces cerevisiae* cells. Can J Microbiol 51:833–839

Valera MJ, Boido E, Ramos JC, Manta E, Radi R, Dellacassa E, Carrau F (2020) The Mandelate pathway, an alternative to the phenylalanine ammonia lyase pathway for the synthesis of benzenoids in ascomycete yeasts. Appl Environ Microbiol 86:e00701–e00720

Van Dijken JP, Scheffers WA (1986) Redox balances in the metabolism of sugars by yeasts. FEMS Microbiol Rev 1:199–224

Voelker AL, Miller J, Running CA, Taylor LS, Mauer LJ (2018) Chemical stability and reaction kinetics of two thiamine salts (thiamine mononitrate and thiamine chloride hydrochloride) in solution. Food Res Int 112:443–456

Vuralhan Z, Luttik MAH, Tai SL, Boer VM, Morais MA, Schipper D, Almering MJH, Kötter P, Dickinson JR, Daran JM, Pronk JT (2005) Physiological characterization of the *ARO10*-dependent, broad-substrate-specificity 2-oxo acid decarboxylase activity of *Saccharomyces cerevisiae*. Appl Environ Microbiol 71:3276–3284

Wang J, Cai R, Xu J, Liu Z (2005) Study on the effect of thiamine on the metabolism of yeast by intrinsic fluorescence. J Lumin 20:216–219

White RL, Spenser ID (1982) Thiamin biosynthesis in yeast. Origin of the five-carbon unit of the thiazole moiety. J Am Chem Soc 104:4934–4943

Wightman R, Meacock PA (2003) The *THI5* gene family of *Saccharomyces cerevisiae*: distribution of homologues among the hemiascomycetes and functional redundancy in the aerobic biosynthesis of thiamin from pyridoxine. Microbiology 149:1447–1460

Wolak N, Kowalska E, Kozik A, Rapala-Kozik M (2014) Thiamine increases the resistance of baker's yeast *Saccharomyces cerevisiae* against oxidative, osmotic and thermal stress, through mechanisms partly independent of thiamine diphosphate-bound enzymes. FEMS Yeast Res 14:1249–1262

Xing H (2007) Impact of thiamine and pyridoxine on alcoholic fermentations of synthetic grape juice, Doctoral dissertation, Washington State University

Yang PF, Pratt DE (1984) Antithiamin activity of polyphenolic antioxidants. J Food Sci 49:489–492

Zeidler J, Sayer BG, Spenser ID (2003) Biosynthesis of vitamin B1 in yeast. Derivation of the pyrimidine unit from pyridoxine and histidine. Intermediacy of urocanic acid. J Am Chem Soc 125:13094–13105

Ziro S (1955) Thiamine uptake by yeast cells. J Biochem 42:27–39