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Review

Characterization and Role of Sterols in *Saccharomyces cerevisiae* during White Wine Alcoholic Fermentation

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Abstract: Responsible for plasma membrane structure maintenance in eukaryotic organisms, sterols are essential for yeast development. The role of two sterol sources in *Saccharomyces cerevisiae* during wine fermentation is highlighted in this review: ergosterol (yeast sterol produced by yeast cells under aerobic conditions) and phytosterols (plant sterols imported by yeast cells from grape musts in the absence of oxygen). These compounds are responsible for the maintenance of yeast cell viability during white wine fermentation under stress conditions, such as ethanol stress and sterol starvation, to avoid sluggish and stuck fermentations.

Keywords: ergosterol; phytosterols; sterol starvation; wine yeast; yeast membrane; oenology

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1. Introduction

The lipidome of eukaryotic cells consists of hundreds to thousands of lipid species that integrate membranes, store metabolic energy, and act as bioactive molecules [1,2]. The lipid classification system, as recently described by Mbuyane et al. [3], consists of eight classes of lipids, i.e., fatty acids, glycolipids (glycerolipids and glycerophospholipids), sphingolipids, prenol lipids, polyketides, saccharolipids, sterols and their derivatives [3,4].

Sterols are part of this eukaryotic lipidome diversity and are essential for the maintenance of cell membrane integrity and optimal functionality. They are mainly responsible for regulating the fluidity, rigidity and permeability of cell membranes, and are surrounded by proteins and phospholipids and protected by a sphingolipid head [5,6]. In mammals, the main sterol is cholesterol, while ergosterol is preponderant in fungi. Phytosterols are plant sterols, the major examples being β-sitosterol, stigmasterol and campesterol [7–9].

Yeasts are able to synthesize, assimilate and accumulate significant amounts of sterols, which are associated with their growth, metabolism and viability during alcoholic fermentation [10,11]. Several yeast species can be used in winemaking, depending on their fermentation capacity and the organoleptic characteristics desired in the wine. *Saccharomyces cerevisiae* is predominantly the first choice of winemakers, due to its good fermentation capacity and resistance to high concentrations of ethanol and sulfur dioxide and low pH. In addition, *Saccharomyces cerevisiae* is a model organism for the study of the molecular organization and regulation of the eukaryotic lipidome [12–15].

Yeasts are unable to synthesize their own sterols under anaerobiosis [16,17]. However, they are capable of assimilating sterols from grape must to restore growth, as the solid particles of grape must are rich in lipids, in particular phytosterols (the lipid fraction of grapes) [7,18]. The clarification step, often used before fermentation during white wine production, decreases the levels of aldehydes and herbaceous alcohols in the final product by removing these particles [19,20].

On the other hand, excessive clarification leads to the development of undesirable organoleptic characteristics and to incomplete fermentation due to the lack of lipids [21,22]. The addition of solid particles rich in lipids is therefore often implemented [23].

Wine alcoholic fermentation depends on the interactions between the yeast strain; the availability of nutrients, such as nitrogen, vitamins, lipids and sugars in the grape must; and the regulation of key fermentation parameters, such as temperature and oxygen. During fermentation, yeasts are exposed to stress conditions, such as an acidic pH (between 2.8 and 3.8) [24], high sulfite concentrations, anaerobiosis [25], and the accumulation of toxic components, such as acetaldehyde, acetic acid, and ethanol [26]. Moreover, nutritional deficiencies and imbalances in grape must can also lead to other forms of stress.

Sterols, as well as fatty acids, are essential for yeast adaptation to fermentation stressors, such as high sugar levels and ethanol toxicity, to avoid sluggish and stuck fermentations [27–30]. Indeed, the requirements for ergosterol and unsaturated fatty acids become more important during fermentation in stress conditions [28,31–33].

The importance and role of lipids in yeast metabolism are well known and were recently reviewed [3,34]. However, there are no recent reviews focused on the role of sterols wine fermentation. In this context, this paper provides an overview of the existing knowledge on the role of sterols in yeast during white wine alcoholic fermentation.

First, the characterization and importance of phytosterols and ergosterol for yeast cell survival will be highlighted. Then, the review will focus on the importance of sterols in stress conditions during oenological fermentation with *Saccharomyces cerevisiae*. Finally, the possibility to compensate for sterol deficiency and the effect of sterol limitation on flavor compounds will be discussed.

2. Importance of Sterols in Yeast Cell Survival

2.1. Composition, Structure and Source

Cyclopentanoperhydrophenanthrene, also termed gonan, is the basic structure of steroids. It is composed of three fused cyclohexane rings and one cyclopentane ring. Steroids that contain at least one hydroxyl group on carbon 3, one unsaturated bond in C-5,6 and that do not have a carbonyl group are termed sterols [35]. These non-polar molecules are essential lipid constituents in animals, fungi and plants.

Ergosterol is the main sterol in fungi (Table 1). It is the equivalent to mammalian cholesterol, as both correspond to the end product of the sterol biosynthetic pathway [6,36]. These two types of sterols have a structure with four rings, an acyl side chain and a hydrophilic hydroxyl group. Nevertheless, ergosterol differs from cholesterol by the presence of two additional double bonds between C7 and C8 in the ring and between C22 and C23 in the side chain [37].

Table 1. Main sterols found in eukaryotic cells.

Eukaryotes	Mammals	Fungi	Plants
Sterol	Cholesterol	Ergosterol Zymosterol Fecosterol Episterol Lanosterol	β-sitosterol Stigmasterol Campesterol

Figure 1 shows the molecular structure of cholesterol compared to that of ergosterol. Ergosterol is produced by yeasts in the presence of oxygen and corresponds to 90% of the total content of sterols for *Saccharomyces cerevisiae* strains and 12% mol of their lipidome [38,39]. Small amounts of intermediates in the sterol biosynthetic pathway, such as zymosterol, fecosterol, episterol and lanosterol, are also yeast sterols [40].

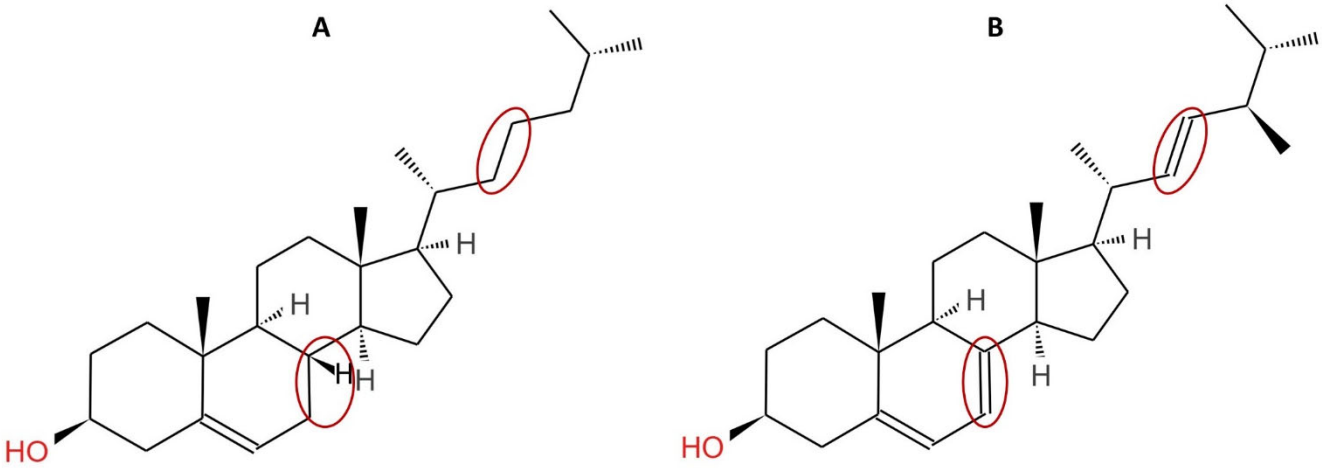

Figure 1. Molecular structure of cholesterol (**A**) and ergosterol (**B**). Differences between molecular structures are circled in red [41].

Phytosterols are sterols synthesized by plants and are notably found in grape berries. The phytosterol content is variable depending on the grape's genetics, growth conditions, tissue maturity and the post-harvest conditions [42]. Among more than 200 phytosterol components detected in plants, the most common are β-sitosterol, stigmasterol and campesterol. In grape berries, β-sitosterol represents between 85 and 90% of the total sterol content [9]. The structure of these phytosterols is very close to that of cholesterol, but differences are observed in the alkyl side chain [43], depending on the phytosterol (Figure 2).

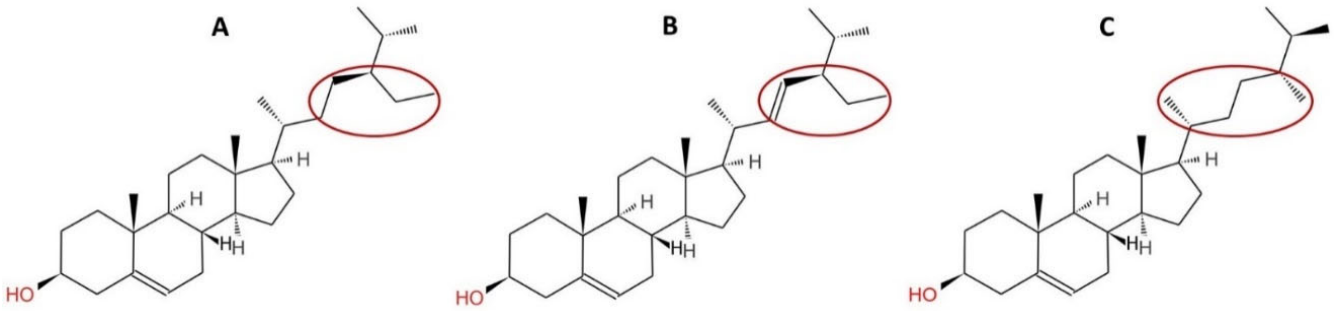

Figure 2. Molecular structure of the main phytosterols in grape berries: β-sitosterol (**A**), stigmasterol (**B**) and campesterol (**C**). Differences between molecular structures are circled in red [41].

2.2. Location and Role

As shown in Figure 3, eukaryotic membranes are characterized by a lipid bilayer with a 7.5 nm thickness, composed of lipids and membrane proteins [16]. Sterols, along with phospholipids, sphingolipids and glycerolipids, are the major lipid components [10]. Sterols' main role is to regulate membrane permeability and fluidity, the absorption of exogenous sterols under anaerobiosis and cell oxygen consumption under aerobic conditions. They are also essential for vital processes, such as vesicle formation and protein sorting, ensuring the viability of eukaryotic cells [5,44,45]. Furthermore, these lipid components are energy sources, signaling molecules and mediators of membrane fusion and apoptosis [46,47].

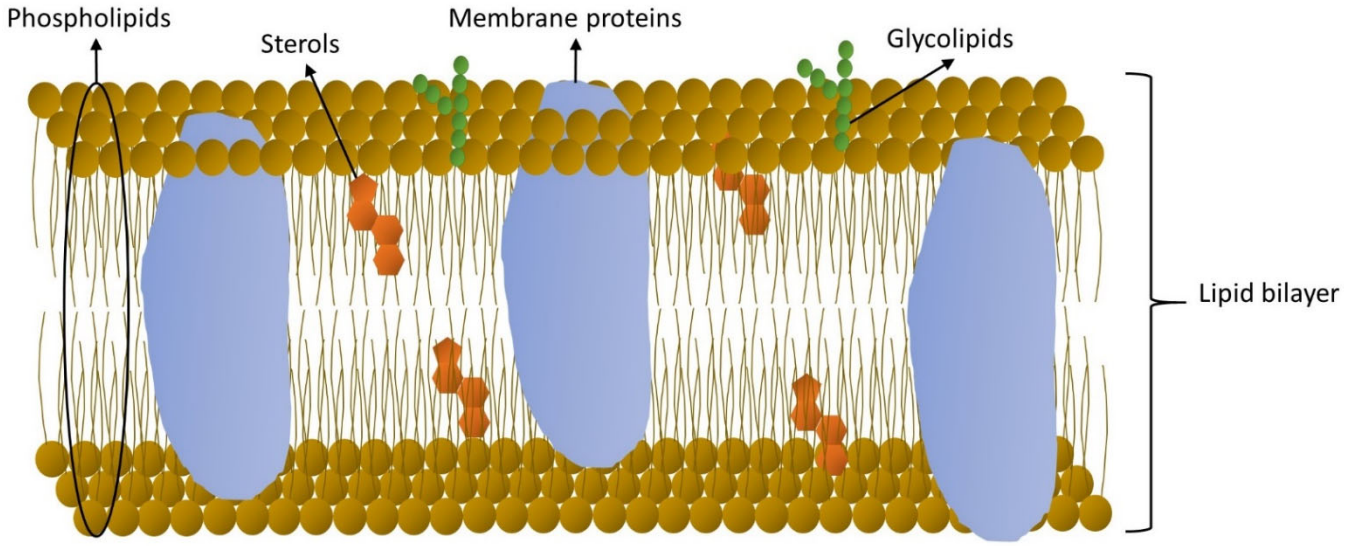

Figure 3. Lipid bilayer of eukaryotic membranes and its components: phospholipids, sterols, membrane proteins and glycolipids.

Yeast cell sterols are mostly found in the plasma membrane, although they are also present in secretory vesicles and lipid particles [40,48]. Ergosterol is the main sterol, followed by other sterols, such as zymosterol, fecosterol and episterol [40]. They are required for membrane structuring, the initiation of cell growth, and the regulation of gene expression [49–51].

The plasma membrane of *Saccharomyces cerevisiae* is composed of functional compartments, known as microdomains, formed by the association of sterols and sphingolipids with proteins. In the yeast membrane, lipid rafts are a class of these domains that are rich in ergosterol. They are composed of proteins that control Na+, K+ and pH homeostasis and the stress response, influencing yeast cell growth and death [52].

Moreover, these domains allow for the maintenance of the liquid-ordered membrane state and are therefore responsible for the regulation and alteration of membrane characteristics [53,54]. The lipid order and a reduction in their fluidity were observed after ergosterol addition in all-atom and coarse-grained molecular dynamics simulations by Ermakova and Zuev [55]: the rigidity of ergosterol rings reduced the mobility of the neighboring acylated lipid chains, which reduced the surface area occupied by lipids and their mobility in the bilayer. Consequently, ergosterol allowed membrane compaction, as well as an increase in its thickness [55].

Interestingly, the deletion of genes from ergosterol biosynthesis has also been shown to have a crucial effect on plasma membrane integrity and dynamics, such as membrane hyperpolarization, protein compartmentation and a decrease in membrane rigidity [56–59]. In addition, yeast cells lacking Pdr18, a plasma membrane ABC transporter, accumulated lower levels of ergosterol [60]. As a consequence, their plasma membrane became more permeable and less ordered, and an increase in cell rigidity was observed [60].

In plants, phytosterols are essential for the regulation of membrane fluidity and permeability, as well as for their metabolism. Silva et al. [61] showed that β-sitosterol and stigmasterol are able to contribute positively to membrane fluidity, owing to a more compact ordered liquid phase. Otherwise, phytosterols control membrane transport and the activity of membrane proteins, such as enzymes, receptors and signal transduction components. As precursors of bioactive steroids, they are also used for the synthesis of secondary metabolites [62–65].

2.3. Sterol Synthesis, Metabolism Storage and Transport

2.3.1. Sterol Synthesis and Metabolism

In yeasts, sterol biosynthesis allows for the maintenance of the plasma membrane and also of mitochondrial morphology [66]. It depends on oxygen and biosynthetic enzymes (mostly Erg proteins) [15,67,68]. The sterol biosynthetic pathway can be divided into three parts: (1) the mevalonate pathway, which takes place in the mitochondria and vacuole; (2) the farnesyl pyrophosphate (farnesyl PP) pathway in the vacuole; and (3) the late pathway in the endoplasmic reticulum (ER) [15,67], as shown in Figure 4. The final product for the late pathway is ergosterol, while its precursors are found in yeast cells in low amounts [6]. In order to produce an ergosterol molecule, 16 NADPH and 24 ATP molecules are consumed [68].

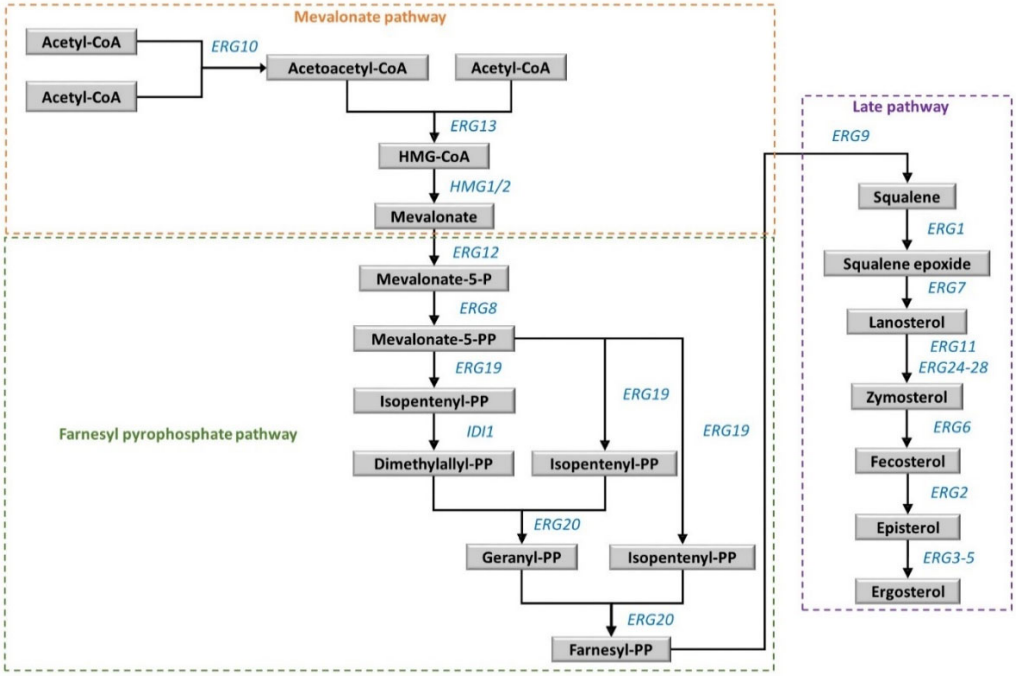

Figure 4. Biosynthesis of ergosterol in yeasts: mevalonate pathway in orange, farnesyl pyrophosphate pathway in green and late pathway in purple. Compounds involved in ergosterol biosynthesis are in black and genes are in blue.

The starting point for the mevalonate pathway is the condensation of two molecules of acetyl-CoA by Erg10p, in order to form acetoacetyl-CoA (Figure 4) [69]. Thereafter, Erg13p condenses this compound with another acetyl-CoA molecule to obtain 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA); this is then reduced to mevalonate by Hmg1p and Hmg2p [70,71]. The farnesyl PP pathway starts with two phosphorylation steps: the first step to synthesize mevalonate 5-P and the second to produce mevalonate-5-pyrophosphate; these reactions are catalyzed by Erg12p and Erg8p, respectively. The action of the mevalonate pyrophosphate decarboxylase Erg19p results in isopentenyl pyrophosphate (isopentenyl-PP) synthesis, which is converted to dimethylallyl pyrophosphate by Idip1p. This precursor is thereafter condensed with a second molecule of isopentenyl-PP to synthesize geranyl pyrophosphate, and then a third molecule to obtain the final product of this pathway—farnesyl pyrophosphate—using Erg20p [15].

The late pathway starts with squalene synthesis in the presence of Erg9p, which is converted to epoxy squalene by the Erg1p epoxidase in the presence of oxygen [56,67]. Thereafter, the lanosterol synthase Erg7p produces lanosterol, which is demethylated, reduced and desaturated to zymosterol by the action of the enzymes Erg11p and Erg24-28p [15]. This component is converted to fecosterol and then episterol by Erg6p and Erg2p, respec-

tively. Finally, the latter compound is desaturated and reduced to ergosterol by the actions of Erg3p, Erg4p and Erg5p [15,72].

Yeast requires oxygen for sterol synthesis, as Erg1p, Erg11p, Erg25p, Erg3p and Erg5p are O_2-dependent enzymes [15]. The regulation of transcription, translation and post-translation mechanisms is thus essential for the maintenance of sterol levels in yeast cells [73]. The transcription factors Ecm22p and Upc2p activate the transcription of sterol synthesis enzymes when yeast strains experience sterol deficiency by binding to the regulatory element of sterols in the promoter region [74]. The endoplasmic reticulum-related degradation pathway is responsible for recognizing the levels of lanosterol and oxysterol. When an excess of lanosterol is detected, Erg1p is degraded by the ubiquitin ligase Doa10p [75]. HMG-CoA reductase (HMGR) degradation also helps to prevent sterol accumulation [76].

2.3.2. Sterol Storage and Transport

An excess of free sterols in the cell can be toxic. To prevent this, the level of sterols in yeast cells is regulated, and any excess sterols are stored in the cell in lipid droplets as steryl ester or secreted into the medium as sterol acetate. Specific lipases are involved in the release of these sterols, depending on the balance between the synthesis, transport and esterification of free sterols, essential for the maintenance of sterol homeostasis [77,78]. The biogenesis of lipid droplets is controlled by the TOR and SNF1/AMPK pathways [79]. Moreover, another alternative consists of the acetylation of these sterols and the deacetylation of sterol acetates by the alcohol acetyltransferase Atf2p and the sterol deacetylase Say1p, respectively [80]. The acetylated sterols are then transported by pathogen-related yeast (PRY) proteins to the plasma membrane, where the highest concentration of yeast cell sterols is found [40,81].

ATP-dependent vesicular and non-vesicular transport pathways transport sterols synthesized in the endoplasmic reticulum to the plasma membrane [18,82]. The insoluble lipid compounds could likely be transported by contact with the membranes of organelles or by transport proteins, capable of solubilizing sterols during transport. In yeast, seven proteins have this function, i.e., the Osh proteins, mainly located on membrane contact sites. The absence of these proteins leads to cell death, while the absence of Osh functions changes the distribution of sterols within cells [83,84]. Osh6p and Osh7p regulation is mediated by the ATPase Vps4p [85].

In addition, other proteins encoded by the *ARV1* gene are also involved in sterol transport, as well as in the absorption of exogenous sterols. When this gene is deleted, sterol levels in the ER and vacuolar membranes increase and sterol incorporation into the plasma membrane is reduced [86]. On the other hand, the deletion of the *NCR1* or *NPC2* genes, associated with the transport enzymes Ncr1p and Npc2p, respectively, has no impact on the distribution of sterols in yeast cells [87–89].

The non-vesicular transport of sterols to membranes is achieved by lipid transfer proteins (LTPs). LTPs are also capable of facilitating the exchange of sterols between the plasma membrane and the ER from membrane contact sites, depending on the sterols' affinity with their lipid rafts [90,91]. Aus1p might be able to facilitate the capture of sterols by LTPs [78]. The Yeh1p, Yeh2p and Tgl1p hydrolases involved in the release of sterols located in lipid droplets are responsible for regulating free ergosterol levels in the cell [92]. The Arv1p membrane protein becomes essential under conditions where sterol esterification is not possible. Therefore, growth difficulties are observed for those strains unable to synthetize this protein [86].

ER membrane homeostasis is also dependent on Apq12p, as this protein allows yeast to grow at a low temperature, regulates mRNA export and ensures nuclear membrane flexibility [93]. Brr6p is mainly responsible for assembling functional nuclear pores, which allows for the esterification of free sterols [94]. Moreover, these proteins may be associated with lipid synthesis [95]. Figure 5 shows the pathways, cellular compartments and proteins associated with the transport of sterols within yeast cells.

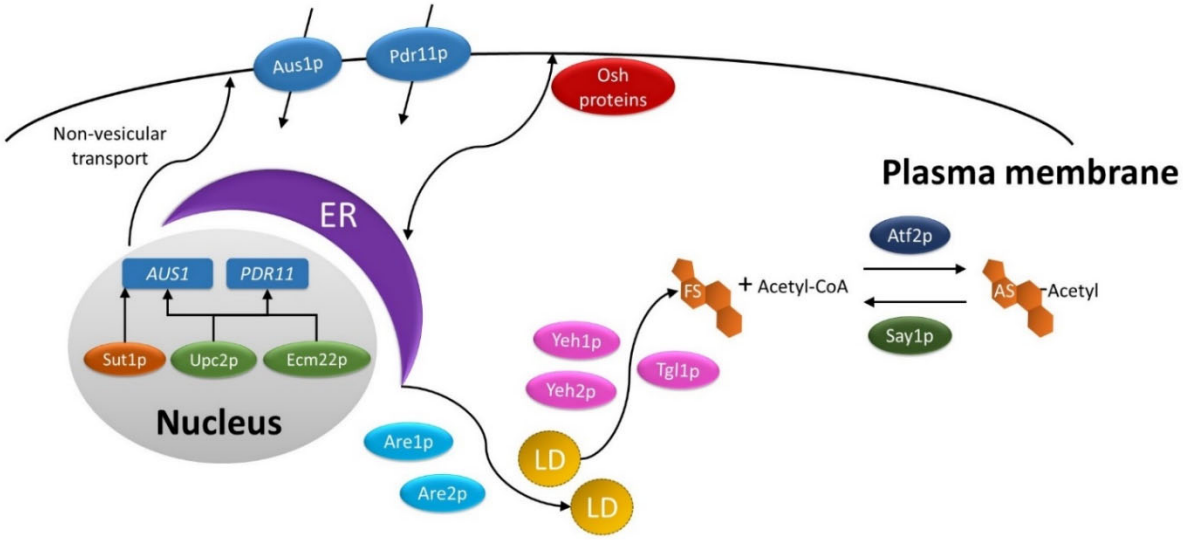

Figure 5. Pathways for the intracellular transport of sterols in yeast. Arrows indicate sterol transport pathways. Major cellular compartments are indicated: plasma membrane; endoplasmic reticulum (ER); nucleus; and lipid droplets (LD). Sterol transport proteins are represented by ABC transporters Aus1p and Pdr11p and Osh lipid binding proteins; Are1p/Are2p acyltransferases convert free sterols into steryl esters (stored in lipid droplets); Yeh1p/Yeh2p/Tgl1p steryl ester hydrolases control the release of sterols located in lipid droplets; Atf2p acetyltransferase for FS (free sterol) acetylation; Say1p deacetylase for AS (acetylated sterol) deacetylation. Transcriptional regulators Ecm22p and Upc2p induce the expression of *AUS1* and *PDR11* and Sut1p of *AUS1* under a deficiency in sterol uptake.

2.4. Phytosterol Assimilation

External sterol sources are not assimilated by yeast under aerobic conditions, as Mot3 and Rox1 inhibit the expression of sterol uptake genes (*UPC2* and *ECM22*) [15]. Concurrently, in presence of oxygen, cell wall properties may also prevent exogenous sterols from reaching the plasma membrane [96]. In contrast, under anaerobiosis, *UPC2* and *ECM22* genes are activated, as the heme-dependent transcription factor Hap1 represses *ROX1* and *MOT3* [15]. Phytosterols are then consumed, allowing for the maintenance of cell growth. They can be directly incorporated into the plasma membrane by flip-flop mechanisms or be esterified and then stored in lipid droplets [96,97].

The absorption of phytosterols involves two ABC transporters (ATP-binding cassettes) located in the plasma membrane: Aus1p and Pdr11p [90,98–100]. Furthermore, these transporters are able to modify membrane properties for the insertion of sterols into the outer leaflet, their flip-flop across the bilayer, or their extraction from the cytoplasmic leaflet [90]. The expression of *AUS1* is induced by the transcription factor Sut1p [101]. Mutant cells that lack the Pdr11p and Aus1p transporters or the Dan1p cell wall protein are not able to take up and esterify exogenous sterols in the absence of oxygen, highlighting the importance of ABC transporters in the flow of sterols [90,100]. This is an advantage of *Saccharomyces* strains, since non-*Saccharomyces* strains are unable to import exogenous sterols, as they do not harbor Aus1p and Pdr11p transporters [102].

3. Impact of Sterols in White Wine Alcoholic Fermentation

Next, the impact of sterol starvation in wine fermentation medium (mostly synthetic medium inoculated with pure *S. cerevisiae* strains in high quantities) will be discussed. First, the importance of sterols to avoid stuck and sluggish fermentations (characterized as long fermentations that are difficult to complete) will be presented. Then, their influence on fermentation kinetics, cell viability, biomass production, nitrogen assimilation and the production of central carbon metabolism (CCM) metabolites will be detailed. The positive effect of sterols on ethanol stress and low temperature resistance and the strategies used to

compensate for the lack of this lipid nutrient, such as the addition of oxygen, solid particles or dry yeasts, will also be presented. In the end, the effect of sterols on aroma production will be highlighted.

3.1. Sterol Starvation

A lack of lipids in the fermentation medium results in sterol stress and induces the production of metabolites from lipid biosynthesis, such as acetic acid and medium chain fatty acids [103,104]. In particular, a lack of sterols and saturated fatty acids makes it difficult for cells to multiply [11,105]. Furthermore, anaerobiosis prevents ergosterol biosynthesis, which has an impact on membrane functionality and cell viability [15,106]. Under anaerobic conditions, the sterols present in the medium are incorporated into the plasma membrane, and, subsequently, into intracellular membranes [107]. From 2 to 8 mg/L of phytosterols (depending on the strain used) are necessary for normal yeast growth at the beginning of alcoholic fermentation [22,108].

A shortage of sterols, fatty acids and oxygen leads to low viability due to the accumulation of large amounts of squalene in cell membranes [109–111]. In these specific conditions, the fermentation time is very long, and, in some cases, this results in incomplete fermentations [22,98].

Da Costa et al. [112] evaluated *S. cerevisiae* fermentation parameters under anaerobic conditions with and without the anaerobic factors (AFs) ergosterol and oleic acid. Cell growth was much lower without the AFs, despite a residual growth due to the presence of a reserve of sterols in commercial active dried yeast strains [113]. Ergosterol was the major neutral lipid found in yeast cells under aerobic and anaerobic conditions with AFs, whereas squalene and lanosterol (ergosterol precursors) were predominant in anaerobic conditions without AFs. In addition, a significant drop in viability was observed under anaerobic conditions without AFs (no living cells after 2 h) [112].

Some authors evaluated the growth and fermentation performances of *S. cerevisiae* under sterol deficiency and their impact on nitrogen metabolism during alcoholic fermentation in a synthetic medium [114,115]. Tesnière et al. [114] showed a better cell viability at the end of fermentation when the nitrogen level was low, while its excess favored cell death. For Duc et al. [115], a limited ergosterol content led to incomplete nitrogen assimilation, losses of viability and incomplete fermentations. Moreover, cells were not able to accumulate glycogen and to develop resistance to thermal shock in this condition.

In the case of sterol starvation and anaerobiosis, the cell reacts by inducing those genes involved in sterol biosynthesis and importation: *ERG28*, *ERG26*, *ERG25*, *ERG1*, *ERG11*, *NCP1*, *ERG9*, *ERG3*, *ERG27*, *ERG6*, *ERG2* and *ERG24* for the biosynthesis of ergosterol and *TIR1*, *TIR3*, *DAN1*, *DAN4*, *TIR4* and *TIR2* genes encoding mannoproteins involved in the importation of sterols. Normally, these genes are overexpressed in anaerobic conditions [116–119]. Surprisingly, Duc et al. [120] showed that they were also overexpressed under ergosterol deficiency conditions, showing that their expressions are dependent on sterol availability. In addition, both *AUS1* and *PDR11* genes, involved in the transport of sterols, and the *MCA1* gene, associated with yeast apoptosis (programmed cell death), were strongly expressed [120].

Very few studies have evaluated the impact of the nature of sterols (phytosterols versus ergosterol) on alcoholic fermentation. Luparia et al. [121] studied the effect of phytosterol (90% β-sitosterol, 5% campesterol and 5% stigmasterol) and ergosterol supplementation in fermentations in a synthetic must with *S. cerevisiae*. Incomplete fermentations and low biomass were observed in the absence of sterols under anaerobiosis. Complete fermentations and a higher amount of biomass were observed in the presence of 15 mg ergosterol/L or different doses of phytosterols. Moreover, the assimilation of phytosterols led to an increase in yeast viability at the end of fermentation. The fact that the ergosterol and phytosterols were not provided in the same amounts in this study makes it difficult to compare the effectiveness of these two sources of sterols and to understand their roles. Further studies are needed to confirm the hypotheses that yeasts prefer to incorporate

ergosterol into their cell membranes and store phytosterols, and that ergosterol is more effective than phytosterols in maintaining membrane integrity.

Sterol deficiency impacts yeast metabolism by triggering lipid synthesis. This results in an overproduction of acetic acid and the accumulation of NADPH (Figure 6). Indeed, it increases the flow of pyruvic acid towards the PHD bypass for the production of cytosolic acetyl-CoA, of which acetate is an intermediate [104]. Moreover, α-ketoglutarate, an intermediate in succinic acid biosynthesis, can be consumed for metabolizing amino acids linked to nitrogen–lipid imbalance [22]. As a consequence, a reduction in succinic acid synthesis by the Krebs cycle is observed. Surprisingly, in this condition, glycerol synthesis is increased (which corresponds to a non-correlation with succinate production). A hypothesis to explain this glycerol behavior would be that the synthesis of triglycerides is strongly activated in order to make up for the lack of lipids, which entails the production of L-glycerol 3-phosphate (an intermediate component of glycerol). Therefore, the excessive flow of this component would be converted into glycerol, which would increase its biosynthesis [22].

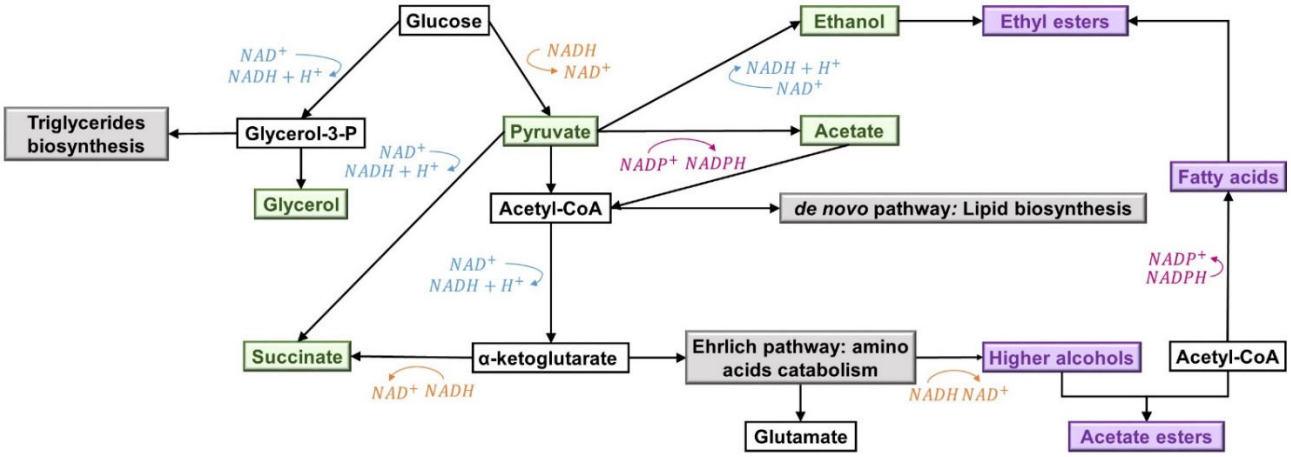

Figure 6. Biosynthesis of CCM metabolites (green), aroma compounds (purple) and associated pathways (gray). Reduction reactions are in orange and pink, oxidation reactions are in blue.

Finally, phytosterols and inactive yeast cell additions in the fermentation medium are capable of increasing sterol availability and reducing the cellular demand for lipids, which entails a decrease in the production of acetic acid [22,122–124].

3.2. Ethanol Stress in S. cerevisiae

Lipids are among the main components of the cell membrane and are essential to resist osmotic stress and high concentrations of ethanol during fermentation. Indeed, membrane fluidity is modulated by adjusting the concentration of sterols and unsaturated fatty acids [125,126].

Ethanol increases membrane permeability and has a negative impact on the transport/retention of protons and essential nutrients, such as amino acids and sugars [28,127,128]. A concentration of 2% *v/v* ethanol is capable of inhibiting 65% of endocytosis by membrane transport proteins, negatively impacting the transport of sugars and amino acids [129,130]. Similarly, 4 to 6% *v/v* ethanol has an influence on protein synthesis [131].

The contact of yeast cells with higher concentrations of ethanol (more than 10% *v/v*) in the medium leads to the diffusion of polar molecules from yeast cells, cellular ATP depletion and a decrease in membrane thickness [128,132,133].

In addition, ethanol modifies the structure and fluidity of the lipid bilayer, increasing the surface area occupied by lipids and resulting in interdigitation [134,135].

Ethanol tolerance in *S. cerevisiae* strains was associated with their lipid content in many studies [136,137]. For example, Lucero et al. [29] showed that strains with high oleic

acid (18:1) and low palmitic acid (16:0) contents were more resistant to ethanol. Likewise, Dinh et al. [30] observed a higher concentration of palmitic acid in strains adapted to high concentrations of ethanol, compared to non-adapted strains, and Alexandre et al. [138] noticed a reduction in oleic acid and an increase in palmitic acid due to ethanol exposure.

Changes in lipid composition is one of the mechanisms developed by cells to survive stress conditions. In fact, yeast membrane fluidity increases when it is constituted by unsaturated fatty acids (as unsaturated bonds decrease the strength of hydrophobic interactions), allowing a greater tolerance of *S. cerevisiae* to stresses linked to temperature and ethanol [31].

S. cerevisiae strains with a higher ergosterol content have also been shown to be more ethanol-tolerant [28,32,139]. Indeed, studies have shown that ergosterol reduces the interdigitation of lipid bilayers in the presence of ethanol [133,140–142]. Interestingly, a decrease in ethanol tolerance was seen for strains deficient in enzymes involved in ergosterol biosynthesis, such as Erg3p, Erg5p or Erg6p [143,144].

These results show that sterols, as well as unsaturated fatty acids, contribute to the ethanol-tolerance of yeast cells, as they maintain optimal membrane thickness in the presence of ethanol, avoiding interdigitation.

3.3. Effect of Temperature on Sterols

The vinification of white and rosé wines at lower temperatures, between 10 and 15 °C, allows a better preservation of volatile aromas, such as higher alcohols and esters [107,145]. However, these temperatures entail longer lag phases and increase the risk of sluggish and stuck fermentations [146]. Indeed, cold stress reduces membrane fluidity and alters the activity of membrane-associated enzymes and transporters [33,107,147].

Redón et al. [33] studied the addition of ergosterol to YPD medium for commercial wine yeast cells grown before their inoculation in a synthetic grape must at 13 °C under anaerobiosis. It was noted that ergosterol favored the synthesis of unesterified fatty acids, increasing membrane fluidity. Moreover, during fermentation, strains that grew in an ergosterol-complemented medium were able to complete fermentation earlier than the control strains grown without sterols [33].

The expression of ergosterol biosynthesis genes, such as *ERG3*, *ERG6* and *IDI1*, is affected by temperature variations [145,148]. Indeed, Δerg3 and Δerg6 mutant strains were not able to complete fermentation at 12 °C, and stuck fermentations were even observed at 28 °C [145]. Better growth was observed in case of *IDI1* overexpression, although delays in fermentation still occurred when *ERG3* and *ERG6* were overexpressed at a low temperature [145].

Saccharomyces strains adapted to low temperature were shown to accumulate less squalene, an ergosterol precursor [149]. Moreover, the accumulation of another ergosterol precursor, fecosterol, and the increased expression of *UPC2* (a gene activating enzymes under sterol deficiency) were both observed in a thermotolerant *S. cerevisiae* strain after either mutation in *ERG3* or *ERG2* deletion [44,59].

These results suggest that the adaptation of yeast to low and high temperatures is associated with the expression of ergosterol biosynthesis genes. Thus, the ergosterol content of yeast cells plays an important role in the adaptation of wine strains during white wine fermentation at low temperatures.

3.4. External Sources: The Addition of Grape Solid Particles, Exogenous Phytosterols, Inactive Dry Yeast Cells and Oxygen

Insufficient dissolved oxygen (lower than 7nM) does not allow adequate sterol synthesis and can provoke a high mortality rate, leading to difficulties in achieving complete alcoholic fermentation, particularly in musts with a low phytosterol content [150–153]. External sources of sterols can then be used to make up for this sterol starvation.

When there is low sterol content inside the cell, yeasts can import phytosterols from the fermentation medium by the production of Aus1p and Pdr11p transporters, induced

by Upc2p and Ecm22p [102,154–156]. The clarification of grape musts leads to a depletion of their phytosterol content [157]. In highly clarified musts, the addition of grape solids decreased the duration of fermentation and exerted a significant and positive impact on yeast cell viability and maximum population [98]. This impact is due to the phytosterol content of grape solids, which allows for the consumption of a greater quantity of assimilable nitrogen by yeasts and a shift from a lipid-limited to a nitrogen-limited situation. The addition of solid particles in such musts is therefore used as a source of lipids to allow better nitrogen uptake, achieve a high yeast biomass and lower the risks of sluggish fermentations [23,98,114]. Similarly, the addition of exogenous phytosterols to the fermentation medium also has a positive impact on the fermentation kinetics, maximum cell population and viability [11,123].

The addition of inactive dry yeasts (IDY) and yeast hulls to a synthetic and a natural must, respectively, with a high nitrogen content and a low lipid content, also allowed an increased biomass, promoting more efficient fermentations due to their ergosterol content [122,158]. Indeed, sterols from IDY can be transferred to active dry yeasts during rehydration. The addition of 150 g/L (dry weight) of IDY during rehydration would correspond to the addition of 40 µg/L of ergosterol to the fermentation medium [158].

In the absence of phytosterols, the addition of 5 to 10 mg/L oxygen permits the restoration of a normal fermentation rate [113,152,159]. Oxygen additions (10 mg/L) at the end of the growth phase made by Ochando et al. [22] in a sterol-deficient synthetic medium mimicking champagne must under anaerobic conditions made it possible to compensate for the lack of lipids through ergosterol and fatty acid synthesis by yeasts, as well as a higher nitrogen consumption. As a result, an increase in maximum fermentation rate, the production of more cells and high viability maintenance were observed, especially for fermentations in must with an insufficient phytosterol content. For the strain studied in this specific work, a concentration of more than 2 mg/L of phytosterols maintained a high viability (more than 80% of living cells) for up to 97% of alcoholic fermentation.

3.5. Effect of Sterols on Aroma Compounds

The production of fermentative aromas during alcoholic fermentation is mostly dependent on the yeast strain [160], assimilable nitrogen [161,162], fermentation temperature [124,163] and must lipid composition [104,164,165]. The nitrogen/lipid balance is a main parameter that influences both fermentation kinetics and the synthesis of fermentation aromas [124].

Medium and long chain fatty acids, higher alcohols, acetate esters and ethyl esters (derived from higher alcohols and fatty acids, respectively) are the main components that contribute to wine fermentative aromas, providing fruity and floral notes [166,167]. Central carbon metabolism (CCM), as well as lipid metabolism and amino acid catabolism by the Ehrlich pathway, have a fairly significant influence on the production of these components [168]. Part of the sugars present in the fermentation medium is directed towards the biosynthesis of CCM metabolites, which contribute to wine sensory aspects: glycerol, acetic acid, pyruvic acid, as well as, in smaller amounts, aldehydes, higher alcohols and their esters (Figure 6).

Supplementation with sterols can increase the production of volatile aroma compounds, such as higher alcohols [124,169–172]. Indeed, a positive correlation between higher alcohol production and sterol content has been observed for ergosterol as well as for phytosterols [124,170,171], as shown in Table 2. One possible explanation would be the repression of alcohol acetyltransferases, responsible for the conversion of higher alcohols to their corresponding esters, in the presence of lipids [173]. However, propanol biosynthesis (a nitrogen marker) is not impacted by the addition of phytosterols [124].

Table 2. Positive impact of sterols (ergosterol and phytosterols) on higher alcohol biosynthesis.

Higher Alcohols	Sterol with Positive Impact on Higher Alcohol Biosynthesis	References
Propanol	Ergosterol	[169,171]
3-ethoxy-1-propanol	Ergosterol and phytosterols	[171]
Isoamyl alcohol	Ergosterol	[169,171]
Isoamyl alcohol	Phytosterols	[124,171]
2-phenylethanol	Ergosterol	[169–171]
2-phenylethanol	Phytosterols	[172]
Propanol and 2-phenylethanol	Phytosterols	[171]
2-methylbutanol and 3-methylbutanol	Ergosterol	[170]
Isobutanol	Ergosterol	[169–171]
Isobutanol	Phytosterols	[124,171,172]

Lipids also impact the production of esters. Indeed, acetyl-CoA (an intermediate in the synthesis of lipids) is associated with both acetate and ethyl ester production. Acetyl-CoA can bind higher alcohols through alcohol acetyltransferases (Atf1p and Atf2p) to form acetate esters [174–176]. Studies have shown that unsaturated fatty acids and oxygen repress *ATF1* expression, despite the fact that the impact of sterols on this gene is not known [171,177–179]. Regarding *ATF2*, its expression should be linked to sterols, as sterol acetylation for the regulation of yeast cell sterol content is mediated by the acetyltransferase Atf2p [80]. Ethyl esters are also associated with lipid metabolism, as the ethanol acyltransferase Eeb1 is capable of esterifying short-chain fatty acids [165].

The impact of sterols on ester production is complex. Varela et al. [170] and Fairbairn et al. [171] noticed an increase in the concentrations of acetate esters and ethyl esters due to sterols (Table 3). Yet, opposite results were found by Rollero et al. [124,164]. A hypothesis to explain the increased content of ethyl esters would be the inhibition of acetyl-CoA carboxylase and thus of long-chain fatty acid formation, which would allow the release of medium-chain fatty acids for the biosynthesis of ethyl esters [175]. Strains with different genetic backgrounds and different fermentation conditions, as well as the varying nitrogen/lipid balance, could explain the divergence of these results.

Table 3. Sterols (ergosterol and phytosterols) and their impact on ester biosynthesis. (+) indicates an increase and (−) indicates a decrease in ester concentration.

	Esters	Sterol and Its Impact on Ester Biosynthesis	References
Acetate esters	Isoamyl acetate	(+) Ergosterol and phytosterols	[171]
	Ethyl acetate, isobutyl acetate, 2-methylbutyl acetate, isoamyl acetate and phenylethyl acetate	(+) Ergosterol	[170]
	Ethyl acetate, isobutyl acetate and isoamyl acetate	(−) Phytosterols	[164,172]
Ethyl esters	Ethyl hexanoate and ethyl octanoate	(−) Phytosterols	[124,164]
	Ethyl acetate	(+) Ergosterol and phytosterols	[171]
	Ethyl propanoate, ethyl butanoate, ethyl hexanoate, ethyl octanoate and ethyl decanoate	(+) Ergosterol	[170]

The sterol content, as well as the strain used and the fermentation temperature, have an impact on the release of varietal aromas in white wines, such as thiols [180,181]. 3-mercaptohexanol (3MH), 4-mercapto-4-methylpentan-2-one (4MMP) and 3-mercapto-hexyl acetate (3MHA) are responsible for the development of fruity citrus, woody aroma and passion

fruit flavors, respectively [182]. According to Saharan et al. [180] and Deroite et al. [104], sterol deficiencies entail the production of low amounts of 3MH and 4MPP and higher amounts of 3MHA. The antioxidant properties of lipid nutrients could explain the reduction in 3MH and 4MMP, whereas the higher levels of 3MHA could result from an increase in the acetylation of 3MH to 3MHA [183,184]. Indeed, the activation of lipid synthesis pathways by the *ATF1* gene stimulates the production of acetyl-CoA, which is acetylated with 3MH to form 3MHA [184].

4. Conclusions

This review integrated the latest findings about sterols in white wine alcoholic fermentation with *S. cerevisiae* strains. We highlighted the key role of sterols in enabling yeast cells to cope with stressful conditions. Indeed, these lipid compounds allow a better nitrogen uptake, leading to higher viability and biomass, and faster fermentations. Interestingly, the significant effect of sterols on yeast physiology also impacts the production and balance of aroma compounds and makes it a major factor of yeast nutrition. However, further studies are required to answer the questions raised in this paper, notably in order to better understand the mechanisms involved in the assimilation of ergosterol compared to phytosterols.

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