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Vitamin content of grape musts and yeast nutrition: A review

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Abstract. The management of yeast nutrition is an essential approach for a better control over wine fermentation process. If the nutritional requirements of yeasts regarding nitrogen were well studied in the last decades, the impact of other molecules, like vitamins, was left mostly unexplored until very recently. A new analytical method developed in the last few years, based on RP-HPLC-UV separation and detection of 8 water-soluble vitamins and their vitamers, allowed the firsts large studies on this thematic. The aim of this paper is to provide an overview of the role of vitamins in oenology, including the most recent advances regarding the vitamin content of grape musts, their significance in yeast nutrition and fermentation performance, and their impact on the production of aroma and sensory related compounds during the fermentation of wine.

1. Introduction

Vitamins are defined as organic compounds distinct from lipids, carbohydrates and proteins, and necessary in small amounts in most species, including wine yeasts, for a properly functioning metabolism [1]. Most vitamins exist in several chemical forms called vitamers [2]. Thus, the term vitamin covers a wide variety of compounds with different chemical structures, some of them being fat-soluble and others water-soluble (Table 1).

Their role in oenology received little attention for decades. Several studies on water soluble vitamins in grape musts were realized between the 1940's and the 1980's [3-11], but they often focused on only one or a few compounds and relied on now outdated analytical methods, such as microbial assays. The key role of vitamins in proper function of yeasts' metabolism suggests they act as major players during alcoholic fermentation of wine, and older studies indeed showed that vitamin deficiencies could lead to sluggish fermentation [12].

The development of a new analytical method by Evers and colleagues [13] allowed further exploration of this thematic in the last few years. This quick review presents the most recent findings that followed, from the vitaminic and vitameric characterization of grape musts to wine yeasts nutritional requirements assessment and an evaluation of the impact of thiamine and biotin on volatile and non-volatile compounds production during wine's fermentation. This paper discusses these recent developments and the perspectives they opened for future research and applications.

Table 1. Water-soluble vitamins, vitamers and abbreviations.

Vitamins	Vitamers	Abbr.
B1 Thiamine	Thiamine	T
	Thiamine monophosphate	TMP
	Thiamine pyrophosphate	TPP
B2 Riboflavin	Riboflavin	RF
	Flavin mononucleotide	FMN
	Flavin adenine dinucleotide	FAD
B3 Niacin	Nicotinic acid	NA
	Nicotinamide	NM
	Nicotinamide adenine dinucleotide	NAD
	Nicotinamide adenine dinucleotide phosphate	NADP
B5 Pantothenic acid	Pantothenic acid	PA
	Coenzyme A	CoA
B6 Pyridoxine	Pyridoxine	PN
	Pyridoxine-5'-phosphate	PNP
	Pyridoxal	PL
	Pyridoxal-5'-phosphate	PLP
	Pyridoxamine	PM
	Pyridoxamine -5'-phosphate	PMP
B7/B8* Biotin	Biotin	B
B9 Folic acid	Folic acid	FA
C	Ascorbic acid	AA

*Both denominations can be found. B8 will be used in this paper.

2. Vitamin content of grape musts

The aforementioned method was derived from an existing method for the analysis of vitamins and vitamers in dried raisins [14]. After adaptation and optimization to the grape must matrix, this method allowed the determination of 19 vitamer from 8 vitamin groups. 85 white grape musts of different varieties, vintages and geographical origins were then characterized [13].

2.1. Vitamin concentration ranges

The concentration ranges of vitamins and vitamers along the different musts proved to be wide, with differences of 10 to 100-fold, depending on the compound, between the less concentrated musts versus the more concentrated ones (Table 2). The concentrations of different vitamins can be widely different in a given must, meaning that a must can be relatively depleted in a specific compound while being relatively rich in other ones. Although the vitamer proportions can vary between musts it seems that some of them are more abundant than other (TPP, NM and PA being the most abundant vitamers of their respective groups for example). Overall, the concentration measured are consistent with what was reported in older studies, and most vitamins can be easily quantified with this method, at the exception of biotin and folic acid, which are present in musts at such low amounts that the measured contents are often below the limit of quantification.

Table 2. Concentration ranges of 19 water-soluble vitamers in 85 white grape musts of diverse origins, varieties and vintages. Adapted from Evers et al. (2023a) [13].

Vitamins		Concentration range (µg/L)	Total vitamin range (µg/L)
B1	T	228 ± 23 - 1079 ± 68	860 ± 40 - 3510 ± 110 *(as T equivalent)*
	TMP	150 ± 12 - 1308 ± 55	
	TPP	258 ± 26 - 2412 ± 72	
B2	RF	48 ± 5 - 2682 ± 129	-
B3	NA	14 ± 2 - 999 ± 17	20 ± 20 - 3240 ± 240 *(as NA equivalent)*
	NM	111 ± 12 - 2860 ± 134	
	NAD	70 ± 7 - 2081 ± 83	
	NADP	137 ± 15 - 2925 ± 181	
B5	PA	91 ± 7 - 2351 ± 197	120 ± 70 - 2700 ± 200 *(as PA equivalent)*
	CoA	63 ± 2 - 1430 ± 37	
B6	PN	44 ± 4 - 2958 ± 27	930 ± 30 - 6940 ± 170 *(as PN equivalent)*
	PNP	107 ± 12 - 2860 ± 34	
	PL	74 ± 6 - 2780 ± 11	
	PLP	540 ± 24 - 2932 ± 150	
	PM	83 ± 8 - 771 ± 79	
	PMP	115 ± 12 - 2321 ± 23	
B8	B	0.5 ± 0.0 – 1.0 ± 0.1	-
B9	FA	0.1 ± 0.0 – 13.3 ± 1.3	-
C	AA	938 ± 100 - 7521 ± 339	-

2.2. Effect of origin, variety and vintage

Since the analysed musts were from different origins, grape varieties and vintages, the authors investigated for potential links between those factors and the measured vitamin contents.

The most significant effect was found for geographical origin. With principal component analysis (PCA) performed on the dataset, followed by the representation on a biplot (Figure 1), the musts formed several clusters by country of origin. While overlapping, these clusters highlighted some trends, with some countries of origin seeming related to higher concentrations of some vitamers (higher PLP being associated with French musts for example). Further analysis showed significant differences for 6 out of the 19 vitamers between Chardonnay musts from France and Argentina. However, it should be noted those results could be unbalanced due to the over-representation of musts of French origin in the dataset.

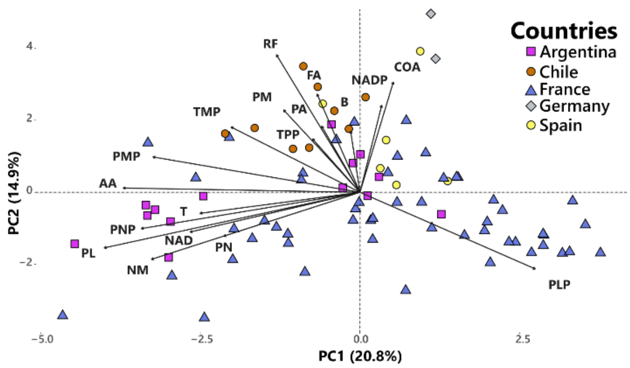

Figure 1. Principal Component Analysis (PCA) biplot of the measured vitamer concentrations, with individual musts labelled according to the country of origin. Adapted from data provided courtesy of M.S. Evers.

Varieties showed smaller effect, with no clear grouping on a PCA biplot being visible. Some varieties formed small clusters, most likely due to the small number of individuals for some cultivars in this dataset. When focusing on 12 musts from the French region of Champagne (4 each from Chardonnay, Sauvignon and Pinot vines), significant differences could be found for 5 out of 19 compounds, suggesting an effect that would need further analyses to be confirmed.

For the vintage no clear effect could be demonstrated as well, with no visible clustering on a PCA biplot and vitamer PL presenting the only significant difference in concentration when comparing 6 Bourgogne Chardonnay musts from vintage 2020 to 6 other from 2021.

Globally those results demonstrated the relevance of this new analytical method and provided updated and more precise data on the vitaminic content of grape musts. They also suggest these molecules, because of their great concentration variability between musts, could be good candidates alongside other compounds for the discrimination of musts depending on grape variety, vintage, origin and potentially other factors. This would, however, require further analysis on larger datasets in order to establish clear trends.

3. Vitaminic nutrition of yeasts

The strong variations in vitamin content between musts and the presence in equivalent quantities of several forms of the same vitamin groups raised the question of the nutritional needs and consumption preferences of common oenological yeasts. These lines of research were therefore explored after the aforementioned work.

3.1. *Saccharomyces cerevisiae*

The first study, performed on 5 *S. cerevisiae* commercial strains [15], showed for the first time the consumption of distinct vitamers by these yeasts. Among the vitamin groups, some vitamers presented different maximal consumption rates, suggesting *S. cerevisiae* might have preferential vitameric sources. This effect is particularly striking for vitamin B3 and B6, with the concentration of vitamers NA, NADP, PM, PMP and PNP dropping sharply at the beginning of fermentation, contrary to the other vitamers of their groups that remained stable or are even released in the media during fermentation.

Further testing on the consumed vitamins highlighted the required character of B1 on fermentation, of B8 for cell growth and of B5 on both aspects (Figure 2). These results are mainly consistent with previous publications [12, 16, 17], but enter in contradiction with some of their results regarding a potential effect of B1 on growth and B8 on fermentation. The fact that strain B showed a reduced growth rate in absence of B1 while no such effect was detected for strain A suggest the exact requirements and the impact of deficiencies could be strain dependant.

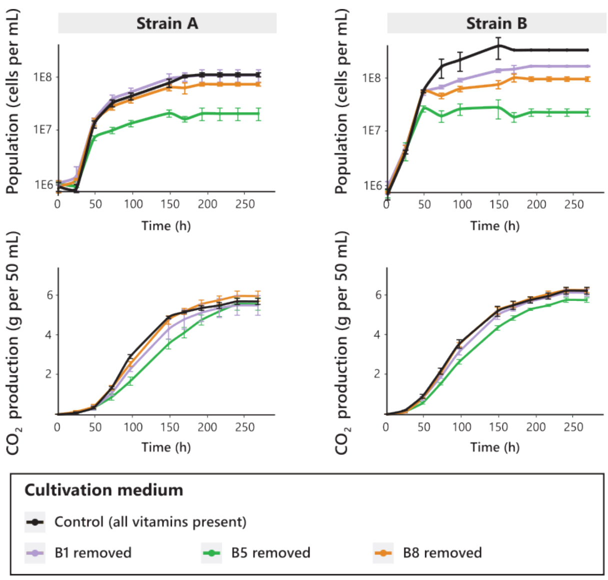

Figure 2. Growth (cell population) and fermentation kinetics (CO_2 production) of two *S. cerevisiae* commercial strains in single-vitamin omission synthetic must media at 20 °C (in triplicate). Results for vitamins other than B1, B5 and B8 are not shown here for a better visibility of the most interesting curves. Adapted from data provided courtesy of M.S. Evers.

Since the most drastic effect was due to B5 removal, the nutritional needs for this vitamin were quantified for 3 commercial strains. When cultivated on synthetic must containing B5 at concentrations ranging from 0 to 750 µg/L, the 3 strains exhibited different behaviours, pointing towards strain dependant threshold concentrations of vitamin B5 for proper growth and fermentation. Overall, the maximum concentration used seemed sufficient for all the strains, and it can be assumed that it would be the case for most wine strains. However, it is worth noting that only 1 out of 4 of the previously analysed natural musts [13] are above this 750 µg/L threshold, which suggests a risk of B5 deficiency, and thus poor fermentation kinetics and cell growth, in most white grape musts.

It should also be noted that in order to be as close as possible to real winemaking conditions, these assays were realised with yeasts in active dry yeast (ADY) form, used according to the manufacturer's specifications. We suspect the initial physiological state of the cells, which may vary depending on the inoculation procedure, could impact the nutritional requirements and the effects of vitamins deficiencies in musts. This could explain some of the inconsistencies between these results and the literature. Another limitation is the synthetic must itself, further experimentation on natural musts should be carried out to confirm the existence and the extent of these requirements.

3.2. Non-*Saccharomyces*

Even though *S. cerevisiae* is obviously the key player of wine fermentation, there is a growing interest of the industry regarding non-*Saccharomyces* (NS) yeasts. Whether they are used for bioprotection [18, 19], reduction of alcohol levels [20], bioacidification [21] or to improve the aromatic profile of wines [22], these yeasts are becoming major players in vinification processes. They are generally used in sequential inoculation or co-inoculation alongside *S. cerevisiae*, which raises the question of possible competition between NS yeasts and *S. cerevisiae* for access to nutrients, including vitamins. This line of research was therefore also explored by Evers and colleagues [23]. With this question in mind, 3 commercial strains were chosen as representants of 3 commonly used NS species (*Starmerella bacillaris*, *Metschnikowia pulcherrima* and *Torulaspora delbrueckii*), and assessed for their nutritional needs in synthetic must medium.

Overall, very different behaviour between species were observed. Regarding the vitamins previously deemed essential for growth and fermentation, NS yeasts behaved quite differently than *S. cerevisiae*. For T and TMP vitamers of the B1 group, if they are indeed consumed by all 3 species, the consumption rates were slower than those of *S. cerevisiae*, and in the case of TPP all species exhibited different comportments: concentration remained stable with *S bacillaris*, decreased with *T. delbrueckii* and raised with *M. pulcherrima*. For B8, another vitamin deemed essential for *S. cerevisiae*, experiments showed consumption by all 3 NS species, with almost total depletion at the end of fermentation in the case of *T. delbrueckii*. Striking differences were also observed for

B5, with both vitamers PA and CoA being produced continuously by *S. bacillaris* while *T. delbrueckii* started consuming PA after an initial release phase at the beginning of fermentation.

The consumption of essential vitamins by some NS yeasts confirms the risks of media depletion and competition for nutrient access with *S. cerevisiae*. On the other hand, some NS yeasts, able to produce essential vitamins and vitamers for *S. cerevisiae*, could be used as a mean to enrich deficient musts. In both case these results constitute useful information for future developments on the management and optimization of sequential and co-inoculation in wine production.

4. Impact of thiamine (B1) and biotin (B8) on volatile and non-volatile compounds production during wine fermentation

Vitamins act as co-factors (or as their precursors) for numerous enzymatic activities in *S. cerevisiae* [24], intervening in the metabolism of amino acids, lipids and carbohydrates. The volatile aromatic compounds of wines largely derive from amino acids and short or medium chain fatty acids [25]. Therefore, it can be assumed that vitamins could have an impact on the volatile composition of wines through their role in the biosynthesis of precursor molecules, thus potentially impacting the aromatic profile of the beverage. This aspect, but also the impact on non-volatile products of the Central Carbon Metabolism (CCM), were explored by Evers and colleagues, with a focus on the effects of B1 and B8 [26].

4.1. Impact on CCM compounds

Fermentations were carried out with thiamine and biotin, each at 3 different concentrations. Out of the 5 CCM products analysed (Acetaldehyde, Acetic acid, D-lactic acid, Glycerol and Ethanol), significant increases in concentration with higher B1 were found for acetic acid and glycerol, while a significant decrease was observed for D-lactic acid.

The same CCM compounds analysed for a B8 effect showed a significant increase in acetaldehyde concentration with higher B8, and interestingly an opposite effect compared to B1 regarding acetic acid and glycerol, with a significant decrease for both in presence of higher B8.

Overall, these results confirm both vitamins play a role on yeast's CCM metabolites production, probably in consequence of their involvement as co-factors for several CCM-related enzymes [24].

4.2. Impact on volatile compounds

Analyses on 31 volatile molecules were carried out on synthetic media fermented in presence of varying concentrations of B1 and B8. Overall, 13 of them showed a significant influence of initial biotin content and 11 a significant influence of initial thiamine. Interestingly, 1 compound was affected by the interaction between both vitamins, but not by them taken individually. Fatty acid metabolites were more affected by B8 than B1, which is consistent with the fact biotin plays an important role in fatty acid biosynthesis in yeast [27]. Among the compounds impacted by B8, 5 were detected in concentrations above their olfactory detection thresholds. In the case of B1, 3 of the impacted molecules were in the same situation.

These results support the idea that both vitamins could be able to modulate the sensory profile of wines, and later experiments followed by sensory analysis confirmed a significant impact of B1 on a synthetic wine olfactory profile [28]. Future fermentation trials using various natural musts instead of synthetic medium and dosed vitamin additions should allow deeper exploration of the potential of vitamins for the modulation of the sensory profile of wines.

5. Conclusion

Vitamins were a blind spot of research in oenology for several decades, only covered by a few publications that were barely scratching the surface of this thematic. However, recent studies have highlighted their essential role in winemaking, and their potential as a new tool for controlling fermentation and the sensory profiles of wines. The analytical method which allowed those results opened new possibilities, and upcoming studies on this subject (trials with more strains, using natural must, and so on) should provide an even better understanding of the importance of vitamins in oenology.

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