

Study of Histidine Degradation and Assessment of Histamine Content in Red Wines During the Malolactic Fermentation

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Abstract: Malolactic fermentation is a secondary fermentation which takes place in wines, leded by lactic acid bacteria, which converts L-malic acid in L-lactic acid and carbon dioxide. Biogenic amines are produced especially by lactic acid bacteria during malolactic fermentation through decarboxylation of amino acids. The amino acids from wines can be decarboxylate resulting biogenic amines: histamine from histidine, tyramine from tyrosine and putresceina from ornithine. In this paper we have monitored the dynamics of histidine metabolism during malolactic fermentation and histamine content was determined before and after malolactic fermentation, in spontaneous and directed conditions. The results of this study shows that when wine pH is high, degradation of histidine is increased and the amount of histamine produced after malolactic fermentation is higher. On the other hand, the results of paper show that increases of histamine content, especially in uninoculated samples of wine with selected malolactic bacteria, when the malolactic fermentation took place spontaneously based on indigenous microflora.

Keywords: malolactic fermentation, histidine, histamine, biogenic amines, malic acid, lactic acid bacteria.

1. Introduction

Amino acids with the largest weight in wine are: proline 570-720 mg/L, arginine 180-450 mg/L, lysine 130-250 mg/L, phenylalanine 76-226 mg/L, glutamic acid 88-98 mg/L, histidine 69-85 mg/L and asparagine 50-56 mg/L [1].

Malolactic fermentation changes content in amino acid of wines. Lactic bacteria have enzymes that contribute to decarboxylation amino acids and formation biogenic amines in wine. Action of lactic bacteria is closely related to the structure of amino acids: arginine amino acid is most affected, followed by histidine, serine, glutamic acid, tyrosine and phenylalanine [2].

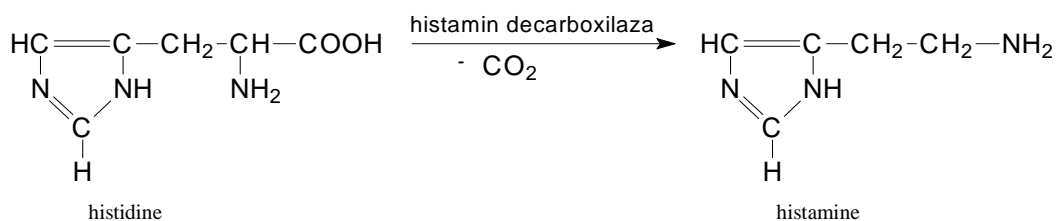
Biogenic amines are produced by malolactic bacteria during the malolactic fermentation process, by amino-acids decarboxilation. In wines there are amino-acids that can be decarboxylated resulting biogenic amines: histamine from histidine, tyramine from tyrosine, putrescin from ornithine. The most important decarboxilases of amino-acids are PLP (piridoxal 5 phosphate) dependent as well as other enzymes involved in amino-acids transformation [3].

The most important reaction of amino acids transformation during malolactic fermentation is the decarboxilation reaction of histidine [4].

Amines abundance after malolactic fermentation is strictly related to microflora but also to amino acids concentration of wines after the alcoholic fermentation. The last ones depend on the must-wine composition, which at her time depends on one hand on the grapes type and on the other hand on the yeasts metabolism [5].

As lactic acid bacteria grows in wine after yeasts, yeasts must have already changed the original composition of the initial must, by using other amino acids and secretion another amino acids during alcoholic fermentation. Furthermore, if the wines are kept in contact with yeasts, lactic bacteria found several amino acids, which are decarboxilated. This explains the high level of amines in some wines [6, 7]. Another reason is variable capacity of lactic acid bacteria for decarboxylation, dependent especially from pH. When the pH is higher, the biogenic amines are produced in higher quantities [8].

With the help of Ridascreen Histamine (Elisa procedure) enzymatic tests, can be determined qualitative and quantitative the histamine from wines.



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In this experiment, histidine and histamine was determined before and after the malolactic fermentation, in wine samples inoculated with commercial preparation INOFLORE R (which contains the *Oenococcus oeni* species) and in wine samples uninoculated, in which the malolactic fermentation took place spontaneously on the inner microflora basis.

2. Experimental

For determination of histidine in wine we were using colorimetric method. We pipette 3 ml of standard solution of histidine, containing $35 \times 1/100$ μ mole (55 μ g) in a 5 mL graduated flask. From a burette 1.2 mL of pyridine was added. After addition, the solution is adjusted to pH 11.0-12.0 with 0.2N NaOH solution. Using a micropipette is inserted 0.04 mL potassium tri-iodide reagent. Shake flask until the contents become homogeneous and appears a stable violet color.

After 30 seconds, check the color formation by adding 0.5 mL of 0.02 N sodium dithionite and was mixed until the solution becomes homogeneous. The volume of flask was adjusted (5 mL) with distilled water, and after 5 minutes, we measure the extinction solution in cell of 1 cm cross-section at the spectrophotometer. The measurement is performed at a wavelength of 560 nm within 15 minutes after the final coloration development [9].

At the determination basis of histamine is an antigen-antibody reaction. After preparing the sample, the histamine is acylated with N-acilhistamine reagent of acylation. In this immune-enzymatic analysis the acylated histamine and free histamine are competing for the bonding places of the antibody. The samples and standards acylated by histamine together with the marked enzymes of histamine are competing for the bonding places of the antibody. The enzymatic conjugate unbend is eliminated by washing.

The enzyme substrate (urea peroxide) and chromogene (tetra-methyl-benzidine) are added in the pails after what they are incubated. The bend enzymatic conjugate

transforms the colourless chromogene into a blue derivat. Adding the stopping reagent (sulphuric acid 0.5 N) leads to the color change from blue to yellow. The reading is made spectrophotometricly at 450 nm. The absorption is backwards proportional with the histamine concentration from the sample [10, 11].

The wines was adjusted at different values of the pH: 3.3, 3.6 and 3.9 with the help of a NaOH 1 M solution. After the sterile filtration of the wines, the wines was poured in bottles of 0.33 L, provided with a digestion-tank.

The malolactic fermentation was induced at 20°C by inoculating in half of INOFLORE bacterial concoction assays which contain *Oenococcus oeni* species. The other half of assays remained inoculated, and the malolactic fermentation was initiated at 20°C, in "spontaneous" conditions, by inner microflora.

3. Results and Discussion

Table 1 presents information on histidine degradation during malolactic fermentation spontaneously (with indigenous microflora) at different pH values. While malic acid degradation occurred only partially histidine was degraded at a rate of 80 % at pH 3.9, but at pH 3.3 histidine was degraded at a rate of 30 %. Also in this table are presented data on histidine degradation during malolactic fermentation directed (using bacterial preparation INOFLORE R). At all values of pH, malic acid degradation was complete before to occur significant degradation of histidine. At pH 3.3, histidine was not significantly degraded (rate of 10 %), while at pH 3.9 the degradation is only at a rate of 50 %.

From the data presented in table 2 it can be noticed that wines, before malolactic fermentation present contents quite reduced of histamine, between 0.4-0.7 ppm. These histamine contents are due yeasts probably which act during the alcoholic fermentation and which posses an enzymatic equipment quite complex containing decarboxilazic enzymes involved in amino acids decarboxilation present in must.

TABLE 1. Histidine degradation of wine during malolactic fermentation at several thresholds of pH

Wine samples	Initial histidine concentration (mg/L)	Histidine concentration after spontaneous FML at pH 3.3	Histidine concentration after spontaneous FML at pH 3.6	Histidine concentration after spontaneous FML at pH 3.9	Histidine concentration after directed FML at pH 3.3	Histidine concentration after directed FML at pH 3.6	Histidine concentration after directed FML at pH 3.9
Oporto	4.260	3.152	1.704	1.022	3.876	3.408	2.343
Merlot	4.375	3.215	1.706	1.006	3.981	3.555	2.362
Pinot noir	5.800	4.234	2.204	1.276	5.220	4.524	3.016
Burgund	7.280	5.278	2.548	1.456	6.406	5.460	3.494
Cadarcă	8.950	6.533	3.132	1.611	7.876	6.712	4.296
Sangiovese	4.875	3.607	1.998	1.170	4.436	3.802	2.681
Blauerzweigelt	7.650	5.202	2.677	1.606	6.732	5.737	3.748
Cabernet Sauvignon	5.930	4.269	2.312	1.541	5.396	4.506	3.202

TABLE 2. Absorbences and histamine concentration values from wine samples before malolactic fermentation

Wine samples	Absorbance read at 450 nm	Histamine concentration ($\mu\text{g/L}$)	Histamine concentration (mg/L)
1. Oporto	1.820	0.850	0.425
2. Merlot	1.779	0.936	0.468
3. Pinot noir	1.744	1.020	0.510
4. Burgund	1.612	1.362	0.681
5. Cadarcă	1.550	1.475	0.737
6. Sangiovese	1.775	0.935	0.467
7. Blauerzweigelt	1.500	1.530	0.765
8. Cabernet Sauvignon	1.730	1.055	0.527

TABLE 3. Absorbences and histamine concentration values from wine samples after malolactic fermentation

Uninoculated wine samples (blank)	Absorbance read at 450 nm	Histamine concentration ($\mu\text{g/L}$)	Histamine concentration (mg/L)
1. Oporto	1.191	2.937	1.468
2. Merlot	1.185	3.112	1.556
3. Pinot noir	1.173	3.143	1.571
4. Burgund	1.156	3.268	1.634
5. Cadarcă	1.123	3.385	1.692
6. Sangiovese	1.177	3.135	1.567
7. Blauerzweigelt	1.109	4.100	2.050
8. Cabernet Sauvignon	1.144	3.311	1.655
Wine samples uninoculated with BMS			
1. Oporto	1.385	2.105	1.052
2. Merlot	1.391	2.362	1.181
3. Pinot noir	1.285	2.518	1.259
4. Burgund	1.249	2.657	1.328
5. Cadarcă	1.213	2.816	1.408
6. Sangiovese	1.306	2.416	1.208
7. Blauerzweigelt	1.192	2.946	1.473
8. Cabernet Sauvignon	1.274	2.559	1.279

From the data presented in table 3, it can be noticed that wines after malolactic fermentation present much consistent concentrations of histamine between 1-2 ppm. These histamine concentration growths during the malolactic fermentation are definitely due the malolactic bacteria which produce histidine-decarboxylases (HDC), which transform the histidine into histamine.

Still, in wine samples with malolactic fermentation there is a difference in what concerns the histamine content. So, for uninoculated wine samples at which the malolactic fermentation occurred spontaneously on the inner microflora basis, the histamine contents are quite high, between 1.468 ppm at Oporto and 2.050 ppm at Blauerzweigelt. Instead, for inoculated wine samples with commercial preparation INOFLORE R (which contains the *Oenococcus oeni* species), the contents of histamine are quite modest, being between 1.052 ppm at Oporto and 1.473 ppm at Blauerzweigelt. This difference of the histamine content between uninoculated (blank) and inoculated wine samples leads us to the assumption that selected malolactic bacteria either can eliminate the indigene lactic bacteria, either can degrade the biogenic amines that could have been produced by some undesired microorganisms.

Still, this experiment comes to clear up another problem. For a long period of time, the oenologists considered only the *Pediococcus* species responsible to for histamine production during the malolactic fermentation.

Nevertheless, the results show histamine concentration increases during the malolactic fermentation even at wines inoculated with *Oenococcus oeni* from which we can conclude that also the *Oenococcus oeni* species can decarboxilate the histidine with a reduced capacity that other lactic bacteria species present in the inner microflora

4. Conclusions

The results of this experiment show that the histamine contents growths are made during the malolactic fermentation especially to wine samples uninoculated with selected malolactic bacteria, at which the malolactic fermentation occurred spontaneously on the inner microflora basis. The histamine concentration in wines with malolactic fermentation is decisively influenced by the wine's microflora (inner or selected), but depends also on the histidine concentration of wines after the alcoholic fermentation. On the other hand, the results of this experiment show that when pH of wine is high (above 3.3), histidine degradation is more intense, and obviously, the amount of histamine produced after malolactic fermentation is higher.

In future, it would be recommendable that also in our country, the content in biogenic amines from wines generally and the histamine content particularly to be

controlled by law. Some countries established maximum limits for the histamine from wines: Switzerland recommends 10 mg/L, Germany recommends 2 mg/L while Belgium and France recommends 5 mg/L and 8 mg/L respectively [12].

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