*Biochemistry*

# Alanine and Aspartic Acid Assimilation by Yeasts During Secondary Alcoholic Fermentation

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**ABSTRACT. Using labelled compounds, the regularity of assimilation and conversion by yeasts of alanine and aspartic acid carbon atoms during secondary alcoholic fermentation was revealed. 76.3% of $1^{14}$C-alanine and 35.5% of $4^{14}$C-aspartic acid, introduced in the fermentation medium were shown to be assimilated and converted by yeasts. Carboxyl carbons of the assimilated compounds were partially oxidized to $CO_2$. The products of assimilation and biotransformation of alanine and aspartic acid were identified in the yeast protein and free amino acids and wine components. By the end of fermentation major part of $1^{14}$C-alanine and $4^{14}$C-aspartic acid conversion products were found in the wine amino acids and organic acids.** *© 2008 Bull. Georg. Natl. Acad. Sci.*

***Key words***: *alanine, aspartic acid, yeast, secondary alcoholic fermentation.*

Study of the physiological peculiarities of yeast cells, manifestation of assimilation and conversion mechanisms of organic compounds, establishment of growth and multiplication regulatory systems are of particular importance for an effective use of progressive technologies for the production of various brands of wine [1, 2].

Formation of sparkling wines involves a set of complicated biochemical and physico-chemical processes the management of which affects the enzyme and chemical composition of such type wines, biochemical characteristics of the yeasts used where one of the key roles is attributed to amino acid exchange [3].

Qualitative and quantitative content of amino acids in wine and their conversion products play a certain technologic role; they are directly or indirectly involved in forming the wine flavour, taste, colour, and to a considerable extent, account for its stability, against turbidity.

The purpose of the present work was to reveal the possible role of alanine and aspartic acid carbon atoms available in the fermentation medium in the synthesis of main yeast and wine components during secondary alcoholic fermentation under extreme conditions.

The industrial strain of wine yeasts *Saccharomyces cerevisiae, var. vini-39* served as a fermentation agent, labelled compounds introduced in the blend had 23.1 MBq radioactivity per litre wine material. Secondary alcoholic fermentation proceeded, as envisaged by classical technologies, in hermetically sealed 0.8 l bottles at $14^0$-$16^0$C. Analysis of the yeast and wine components was made as soon as essential fermentation was over, using conventional chemical, chromatographic and autoradiographic methods. The fermented wine material was released from carbon dioxide, under high pressure, with a specially constructed device with taps. $CO_2$ released from the bottle was gradually chemically linked with 30% KOH. Radioactivity of the yeast and wine components was measured on scintillation spectrometer [4].

The results obtained indicate that carbon atoms of pyruvate family, representative amino acids studied by us – $1^{14}$C-alanine and $4^{14}$C-aspartic acid, are assimilated by yeasts during secondary alcoholic fermentation with varying intensity. Incorporation with varying intensity of amino acids of similar genetic origin in the yeast biomass cannot be explained unequivocally. On the basis of the current achievements in the yeast biochemistry it may be said that given definite $t^0$, pH and $O_2$, the biom-

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ass incorporation intensity is largely determined by the physiological state of yeasts and their biochemical potential, molecular weight of the substance to be assimilated and its structural peculiarities, initial amount of the substance in the fermentation medium, nutrition value, the mechanisms of interstimulation and competitive inhibition of assimilation of the components in nutrition medium. The indicated factors also affect the distribution of substances in separate fractions of biomass and their oxidation intensity.

Experimental evidence shows that, though the dry biomass weights of yeast yielded as a result of fermentation are approximately equal, there is an acute difference in other indices. Alcoholic medium and gradually increasing excessive pressure of carbon dioxide have a certain effect on yeast metabolism [5]. 76.3% of $1^{14}$C-alanine introduced in the fermentation medium is assimilated and converted by yeasts, whereas ass imilation of $4^{14}$C-aspartic acid occurs with twice and lesser intensity. Approximately similar consistency is revealed between the oxidation intensities to $CO_2$ rted $1^{14}$C-alanine is intensively oxidized to $CO_2$ (50%), while oxidation intensity to $CO_2$ of $4^{14}$C-aspartic acid is about 20%.

As to their incorporation in the yeast biomass, there is an opposite picture: incorporation of $4^{14}$C-aspartic acid in the yeast biomass occurs almost 4 times more intensively than of $1^{14}$C-alanine. Study of different aspects of nitrogen metabolism demonstrates that the direct correlative dependence between amino acids' assimilation intensity and the need for their incorporation in protein does not often manifest itself. So that the most readily assimilated amino acids do not form the most important constituent elements of biomass, which is due to quite a number of factors. Therefore, often there is a certain difference between the amino acids' assimilation, i.e. the need and their fixation and nutrition, when they are used in cell synthesis [6].

Of the amino acids examined in our experimental conditions the use of carboxyl carbon of alanine is rather limited for the synthesis of the yeast cell components. Evidence indicates (Table 1) that as a result of $1^{14}$C-alanine conversion only 4 amino acids appeared radioactive in the yeast biomass, both in protein and free amino acids' pool. Among them, in protein as well as in free amino acids pool it is alanine that is distinguished by its high radioactivity. In spite of the fact that assimilation of amino acids in the yeasts is related with decarboxylation and desaminization processes, in our conditions alongside with intensive oxidation of $1^{14}$C-alanine there seems to take place its direct assimilation as well, which is noted especially intensively in the logarithmic phase of yeast evolvement, when the fermentation medium represents a full-value nutrition one. Characteristically, similar consistence was also observed in the results of numerous experiments conducted on Rkatsiteli wine. Apart from the fact that alanine was one of the

Table 1

Distribution of radioactivity (%) during assimilation and conversion of $4^{14}$C-alanine by yeasts

| Radioactivity of amino acids (%) of yeast overall radioactivity | | | | Distribution of wine radioactivity (%) | | | |
|---|---|---|---|---|---|---|---|
| Protein | | Free | | Amino acids | | Organic acids | |
| 82.0% | | 0.1% | | 48.0% | | 52.0% | |
| Distribution of radioactivity (%) in the identified compounds of separate fractions | | | | | | | |
| Alanine | 72.6 | Alanine | 70.8 | Glutamic acid | 17.1 | Lactic acid | 44.4 |
| Lysine | 15.7 | Valine | 19.2 | Threonine | 15.5 | Succinic acid | 20.6 |
| Leucine | 10.0 | Leucine | 6.4 | Leucine | 13.0 | Fumaric acid | 16.9 |
| Valine | 1.7 | Lysine | 3.6 | Asparagine | 11.8 | Glyoxylic acid | 7.7 |
| | | | | Valine | 11.6 | Citric acid | 6.4 |
| | | | | Serine | 11.2 | Malic acid | 3.4 |
| | | | | Aspartic acid | 8.7 | Oxalic acid | 0.6 |
| | | | | Proline | 3.8 | | |
| | | | | X | 7.3 | | |

Table 2

Distribution of radioactivity (%) during assimilation and conversion of $4^{14}$C-aspartic acid by yeasts

| Radioactivity of amino acids (%) of yeast overall radioactivity | | | | Distribution of wine radioactivity (%) | | | |
|---|---|---|---|---|---|---|---|
| Protein | | Free | | Amino acids | | Organic acids | |
| 58.6% | | 0.1% | | 65.9% | | 34.1% | |
| Distribution of radioactivity (%) in the identified compounds of separate fractions | | | | | | | |
| Proline | 28.1 | Valine | 60.0 | Valine | 35.2 | Malic acid | 37.6 |
| Phenyl-alanine | 23.2 | Glutamic acid | 20.0 | Threonine | 21.0 | Succinic acid | 26.8 |
| Asparagine | 12.2 | Leucine | 20.0 | Cysteine | 14.6 | Glyoxylic acid | 24.7 |
| Aspartic acid | 8.5 | | | Glutamic acid | 9.8 | Fumaric acid | 7.2 |
| Glutamic acid | 7.8 | | | Alanine | 5.6 | Citric acid | 0.4 |
| Alanine | 3.5 | | | Methionine | 5.1 | X | 4.1 |
| Serine | 3.5 | | | GABA | 3.7 | | |
| Tyrosine | 3.5 | | | Leucine | 2.7 | | |
| Valine | 3.1 | | | Proline | 2.3 | | |
| Methionine | 3.1 | | | | | | |
| Leucine | 1.2 | | | | | | |
| X | 2.2 | | | | | | |

major ones among free amino acids during nitrogen assimilation in the vine, it appeared to be a perfect donor of amino group at the same time [7].

Most part (97.3%) of $1^{14}$C-alanine assimilation products was found in wine components by the end of secondary fermentation. Radioactivity is almost equally distributed between the fractions of wine amino acids and organic acids. Among wine amino acids the principal ones are glutamic acid and threonine; of organic acids: lactic and succinic acids. Distribution of radioactivity in the identified organic acids clearly shows that their synthesis is related to further conversions of pyruvates obtained from alanine, for which the entire alanine carbon skeleton is used.

35.5% of aspartic acid is assimilated and converted by yeasts during secondary alcoholic fermentation. About 20.0% of $4^{14}$C-aspartic acid is isolated as radioactive carbon dioxide. But as was mentioned, incorporation of $4^{14}$C-aspartic acid in the yeast biomass occurs almost 4 times more intensively than of $1^{14}$C-alanine. Especially broad is the qualitative and quantitative composition of protein amino acids (Table 2). Proline and phenylalanine are distinguished for their extremely high radioactivity among the protein amino acids identified in the biomass. It is remarkable that high radioactivity of proline was noticed in the process of natural alcoholic fermentation too, when $4^{14}$C-aspartic acid was introduced into the fermentable grape juice and the medium content and cultivation conditions were different from those of secondary alcoholic fermentation [8].

In our experimental conditions, $4^{14}$C-aspartic acid conversion products in the yeast free amino acids pool are represented by 3 amino acids. By the end of fermentation, as during $1^{14}$C-alanine assimilation, the main part, more than 90%, is distributed among wine components. Amongst them amino acids and organic acids are the essential ones.

Using radioautographic analysis, 9 amino acids and 5 organic acids are identified. Of amino acids valine, threonine and cysteine are the main ones; of organic acids: malic, succinic and glyoxylic acids.

Distribution of radioactivity among the identified organic acids during conversion of the studied amino acids, representatives of pyruvate family, indicates the functioning of tricarbonic acids and glyoxylate cycle.

Our experimental results indicate that, though the studied amino acids being near by their genesis were labelled in carboxyl, these carbon atoms of alanine and aspartic acid are diversely used by yeasts during secondary alcoholic fermentation, carboxyl carbon of alanine is preferentially oxidized to $CO_2$. $4^{14}$C-aspartic acid is more intensively incorporated in the yeast biomass. By the end of fermentation, however, in both cases the great part of radioactivity is found in the wine components. Carbon dioxide refixation is likely to have a certain role in such diversity of conversion products of the studied compounds.

While considering the results obtained it is necessary to mention that the compounds explored by us are assimilated by yeasts during secondary alcoholic fer-

mentation both by the initial and subsequent generations. It is necessary to bear in mind that the "initial" cell may during fermentation bud off many times and in accordance with cultivation conditions form from 8 to 40 cells. It is also important that the yeast cells have large surface area. Because of this incorporation of nutrition substances in the yeast cell occurs rapidly.

From a biochemical point of view it is rather important that both one and several generations of yeast in the process of secondary fermentation are at different stages of cell cycle development. At the same time, regulation of biochemical processes conducted by yeasts is strongly dependent on cultivation conditions and especially on the medium content. At a separate moment of secondary alcoholic fermentation the qualitative and quantitative composition of the medium, oxidation-reduction potential, temperature, enzyme activity alter dynamically. Therefore, the data obtained must be considered to be a result of the viability of all generations of yeast available in the medium throughout the entire process of secondary alcoholic fermentation, each period of which is due to the viability of definite generations of yeasts most adapted to it.

*ბიოქიმია*

# ალანინისა და ასპარაგინმჟავას ასიმილაცია საფუვრების მიერ მეორეული სპირტული დუღილის პროცესში

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ნაშრომში შესწავლილია მეორეული სპირტული დუღილის პროცესში საფუვრების მიერ ასიმილირებული $1^{14}C$-ალანინისა და $4^{14}C$-ასპარაგინმჟავას ნახშირბადატომების როლი საფუვრისა და ღვინის ძირითადი კომპონენტების სინთეზში. მადუღარ აგენტად გამოყენებული იყო ღვინის საფუვრების საწარმოო შტამი *Saccharomyces cerevisiae, var. vini-39*. მეორეული სპირტული დუღილი მიმდინარეობდა კლასიკური ტექნოლოგიით გათვალისწინებულ ჰერმეტულად დახურულ ბოთლებში $14^0$-$16^0C$-ის პირობებში.

დადგინდა, რომ პირუვატის ოჯახის წარმომადგენელი ამინომჟავების – ალანინისა და ასპარაგინმჟავას ნახშირბადატომები განსხვავებული ინტენსივობით შეითვისება საფუვრების მიერ. საფუვრებმა შეითვისეს და გარდაქმნეს არეში შეტანილი $1^{14}C$-ალანინის 76.3% და $4^{14}C$-ასპარაგინმჟავას 35.5%. ალანინის კარბოქსილური ნახშირბადის 50% $CO_2$-მდე იჟანგება. $4^{14}C$-ასპარაგინმჟავას ნიშანდებული კარბოქსილის $CO_2$-მდე დაჟანგვის ინტენსივობა 20%-მდე აღწევს. ამავე დროს, $1^{14}C$-ალანინთან შედარებით, $4^{14}C$-ასპარაგინმჟავა თითქმის 4-ჯერ უფრო ინტენსიურად ერთვება საფუვრის ბიომასაში. ალანინისა და ასპარანგინმჟავას ბიოტრანსფორმაციის პროდუქტები იდენტიფიცირებულია საფუვრის ბიომასისა და ღვინის კომპონენტებში.

შესწავლილი ამინომჟავების რადიოაქტიურობის უპირატესი ნაწილი დუღილის ბოლოსთვის ღვინის ამინომჟავებსა და ორგანულ მჟავებში გვხვდება. მეორეული დუღილის პროცესში დეზამინირებისა და დეკარბოქსილირების მექანიზმებთან ერთად ფუნქციონირებს ტრიკარბონმჟავების მოდიფიცირებული ციკლი, რომელიც საფუვრის უჯრედში მეტაბოლიზმისთვის საჭირო ნაერთების წარმოქმნას განაპირობებს.

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