

## **DYNAMICS OF ACCUMULATION OF HIGHER ALCOHOL-TYROSOL IN THE PROCESS OF ALCOHOLIC FERMENTATION OF HONEY SWEET**

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### **ABSTRACT:**

**Alcoholic fermentation is a crucial process of making a drink. Honey wine differs from other alcoholic beverages by its specific honey aroma and taste, the organoleptic properties of which determine the quality of the product. Honey wine contains volatile components along with other substances: ethers and higher alcohols, which differ from each other in their organoleptic properties and can have different effects on the taste and aroma of the product. Higher alcohols are derived from amino acids in various ways (derived from raw materials, as well as obtained by chemical and biochemical transformations of various substances); the amino acid tyrosine is converted during the alcoholic fermentation process to form the aromatic alcohol tyrosol. The latter is characterized by the smell of honey and candle. Based on the above, we aimed to study tyrosol in our research facilities. To determine the dynamics of the accumulation of higher**

**alcohol-tyrosol in the process of alcoholic fermentation of honey sweet, we used: paper chromatographic method for qualitative analysis, and spectrophotometric method for tyrosol quantification. It has been found that the most abundant alcohol with the smell and taste of honey - tyrosol, is present in small amounts in honey and is found in relatively large quantities in the product obtained from its alcoholic fermentation - honey wine.**

**KEYWORDS:** Honey, alcoholic fermentation, tyrosyl, tyrosine, higher alcohols.

### **INTRODUCTION:**

The production and development of alcoholic beverages creates new theoretical and practical tasks in the production of alcoholic fermentation, the solution of which requires the study of biochemical and microbiological processes of industrial raw materials that take place during the process of alcoholic fermentation. Alcoholic fermentation is a crucial

process of making a drink. Honey alcoholic beverage is an original beverage that differs from other alcoholic beverages by a specific honey aroma and taste, whose organoleptic properties determine the quality of the product. Honey wine is characterized by volatile components that form a special composition with a defined arrangement of flavors that form a special composition in aroma and taste. The volatile components of honey wine contain honey essential oils and fermentation products. Along with other components, wine contains ethers and higher spirits, which differ from each other in their organoleptic properties and can have different effects on the taste and aroma of the product. It has been established that higher alcohols are derived from amino acids according to the Ehrlich scheme. Although the positive properties of higher alcohols in products obtained by alcoholic fermentation have not yet been fully studied, based on available research we can conclude that they enrich their aroma with characteristic specificity. It is known that the amino acid tyrosine is converted during alcoholic fermentation to form the aromatic alcohol tyrosol [Ehrlich, 1911]. The latter is characterized by the smell of honey and candle.

A yeast cell needs a constant source of energy for activity, which it receives by fermentation as a result of metabolism, i.e. chemical-biochemical transformations of substances. During the alcoholic fermentation, the presence of nitrogenous substances in honey in the area is of special importance. They are essential nutrients for the nutrition and reproduction of yeast. However, it has been established that yeasts from the form of nitrogenous substances use simple nitrogenous substances for food: amides, amino acids and other compounds.

Literary observations have shown that yeast reproduction is stimulated and, consequently, fermentation is activated when

the number of amino acids in the fermentation area is higher (at least two amino acids); The mixture of amino acids accelerates the multiplication of yeast: two amino acids - by 20%, three - by 28% and eight - by 50%.

In addition to the amino acids that affect the multiplication of yeasts, they also interact with alcohols produced in alcoholic fermentation to form a special composition with a definite agreement on taste and aroma.

Aromatic (volatile) components of honey wine contain honey essential oils and other fermentation products. Along with other volatile components, wine contains ethers and higher alcohols, which differ from each other in their organoleptic properties and can affect the taste and aroma of the product in different ways.

The goal of the study was to study the dynamics of the accumulation of higher alcohol -tyrosol in the process of alcoholic fermentation of honey sweet. We used the spectrophotometric method of analysis to solve the problem.

As it is known, alcoholic fermentation is a crucial technological process of making beverages, which involves the joint process of chemical, biochemical and microbiological processes in the fermentation area.

To make honey wine, it is necessary for the honey sugar in the fermentation area to undergo alcoholic fermentation (from sugar with other substances, mainly alcohol and carbon dioxide), as a result of which honey wine is produced, the quality of which depends on the raw material - chemical composition of honey.

Alcohol fermentation does not develop naturally in the desired direction in honey, so it is necessary to prepare honey for alcoholic fermentation, which provides for the reduction of sugar and acidity in the fermentation area in the first place.

The high concentration of sugar in sweets negatively affects the quality of honey wine.

From a low-sugar honey blend, wine is made with richer ingredients than a high-sugar blend. It is known that the dry matter in the chemical composition of natural honey should be at least 78-80%, in which carbohydrates occupy about 75%. The pH of natural honey varies from 4.0 to 5.5, and the acidity does not exceed 0.1-0.7%. The total amount of nitrogenous substances in honey, according to a number of authors, varies from 0.4 to 1.9%. Depending on the geographical location and botanical origin of honey plants in Georgia, nitrogenous substances are found in different amounts in honey samples. The total amount of amino acids in Georgian honey is 0.5-2.48% [Akhvlediani, 1950]. It is known from the literature that 18 amino acids are found in Georgian honey samples. Of these, 9 essential amino acids predominate: methionine, valine, tyrosine, threonine, phenylalanine, leucine, isoleucine and proline [Koblianidze, 1980].

Alcoholic fermentation does not develop in natural honey due to the high osmotic pressure of sugar, so honey sugar should be diluted with water to 18-20% (to the condition of grape sweetness). Grape sweet - a ratio of sugar and non-sugar substances, the recognized standard of the finished alcoholic fermentation product - wine-making juice.

We prepared an 18% honey-sugar mixture for the research objects and produced an alcoholic fermentation to obtain honey wine material according to the method developed by us. During the alcoholic fermentation process, fermentation was observed according to the variability of sugars.

The object of research was:

- 1) Honey samples - according to the geographical location in Georgia and the botanical origin of honey plants;
- 2) Sweet of honey samples (honey - water 1: 4) - an aqueous solution of honey containing 18% sugar and 4 g / l acid;
- 3) Honey wine, the chemical composition of

which is given in Table 1.

Table 1. Chemical parameters of honey wine

No	Name the parameters	Content
1	Ethyl alcohol content, vol. %	11,2
2	Mass concentration of volatile components, g / dm <sup>3</sup>	1,0
3	Mass concentration of titrate acid, g / dm <sup>3</sup>	4,0
4	Total mass concentration of sulfuric acid, mg / dm <sup>3</sup>	15
5	Permissible yeast content, %	2,8

For research methods used in the literature include current technical conditions and standards. The mass fraction of regenerating sugars and sucrose in honey was defined by the standard 19792-87 (natural honey). Invert sugars in aqueous honey were determined by the Bertrand and Soxhlet methods.

According to the literature, the acidity of honey does not exceed 0.1-0.7%. The total acidity does not exceed 0.5 g / dm<sup>3</sup>. This even indicates that the boiling point is the total acidity is lower than normal and the acidity needs to be raised artificially. To increase the acidity, we mostly used citric acid to increase the total acidity of the aqueous solution of honey.

For perfect conduct of alcoholic fermentation of honey mixture and maximum accumulation of aromatic components in the fermented mass, we used cultural yeast IOC 2000, 3% of the total liquid volume.

The fermentation area of the selected honey mixture was fermented with selected sugars, acids and yeast using the classic technology of making dry table wine.

Extracts dry matter, dry matter, titrate and volatile acids, pH, tannins and pectin, total and ammoniac nitrogen, tannins, ethanol, methanol, higher alcohols, acetyls and aldehydes, heavy metals and other components. The experiments

were conducted in the biochemical laboratory of the Agrarian Faculty of the Georgian Technical University.

For the experiment, we prepared an 18% honey-sugar mixture and made an alcoholic fermentation to make honey wine. During the alcoholic fermentation process, fermentation was observed according to the variability of sugars. During the course of the experiment, a particularly noteworthy fact is the qualitative changes in free amino acids in the process of alcoholic fermentation under the influence of pure yeast culture.

In the process of alcoholic fermentation, amino acids undergo transformations and at this time higher alcohols are formed from them. This fact is true of the amino acid tyrosine, which during alcoholic fermentation is converted into a highly aromatic alcohol with a pleasant aroma of tyrosine.

For the experiment, in the aqueous solution of honey and in order to study the amount and accumulation dynamics of aromatic alcohol-tyrosol during its alcoholic fermentation, we took 50-50 ml samples: Honey juice was cooled in a vacuum evaporator, dried with silica gel, eluted with ethyl alcohol, filtered and concentrated before the fermentation and during the alcoholic fermentation period every 48 hours.

The thickened mass was chromatographed (thin-layer chromatography: silica gel plate JIC 5/40 + 13% gypsum, system - chloroform: ethanol (95:5) reagent - diazotized sulfanilic acid). Quantitative determination of tyrosol in the honey mixture and the wine material obtained from it was performed by spectrophotometric method (Prof. Mujiri et al. (1995). We measured the optical density  $\lambda_{\max}$  - 280 nm at wave length. According to the magnitude of the optical density, we constructed a curve on the quantitative content of tyrosol. The variability curve of tyrosol, sugars and ethyl alcohol amount in the alcoholic fermentation

process of an aqueous solution of honey is given in Figure 1.

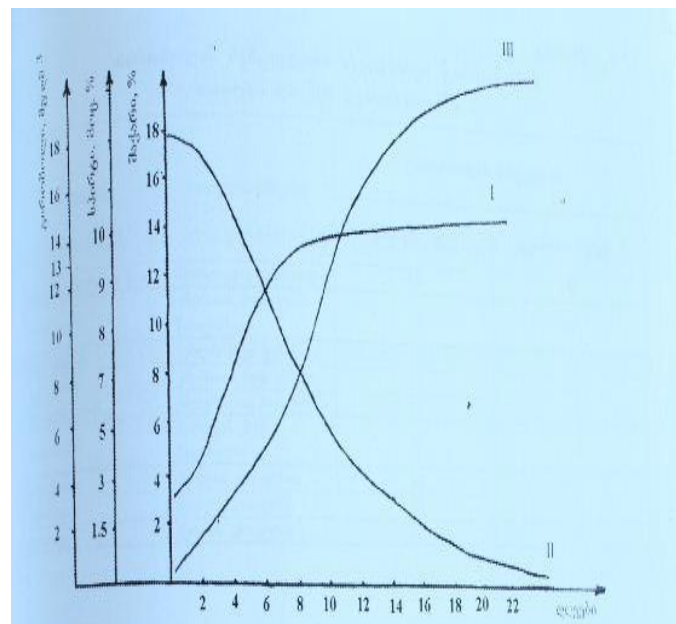


Fig.1. Dynamics of tyrosol accumulation in honey mixture

In the process of alcoholic fermentation

I - tyrosol content curve;

II - sugar variability curve;

III - Alcohol variability curve.

According to experimental data, the determination of the amount of tyrosol in aqueous honey solution and its alcoholic fermentation process showed that its amount in aqueous honey solution is equal to 3 mg/dm<sup>3</sup>, which changes slightly within 48 hours after the start of fermentation, with an increase tendency. During this period the sugar content in the solution is close to 18%. After 48 hours (2-5 days) the amount of tyrosol in the boiling area increases sharply. During the same period there is an intense decomposition of sugar by yeasts and the formation - accumulation of ethyl alcohol. By this time the tyrosol content in the boiling area reaches 13 mg/dm<sup>3</sup>. As for the post-fermentation period, before its final completion, the amount of tyrosol increases slightly and reaches 14.1 mg/dm<sup>3</sup> (see Table 1).

Table 1 Quantitative determination of tyrosol content by optical According to the density,  $\lambda_{\max}$  - 280 nm

Nº	Tyrosol content, $\mu\text{g} / \text{ml}$	Optical density	Nº	Tyrosol content, $\mu\text{g} / \text{ml}$	Optical density
1	1	0.05	9	9	0.24
2	2	0.08	10	10	0.27
3	3	0.11	11	11	0.29
4	4	0.12	12	12	0.33
5	5	0.15	13	13	0.35
6	6	0.17	14	14	0.37
7	7	0.19	15	15	0.39
8	8	0.22	16	16	0.42

The analysis of the results showed that the aqueous solution of honey before alcoholic fermentation contains the higher alcohol - tyrosol. In this way, it differs from grape juice in the process of alcoholic fermentation, in grape juice, as well as in the aqueous solution of honey, tyrosol is formed at the expense of amino acid tyrosine conversion. The data available in the literature tell us nothing on the presence of tyrosol in honey. However, researchers note that some of the higher alcohols in the form of ether are included in the essential oils of the flower and give a pleasant floral aroma both in the free and in the form of ether, which determines the specific taste of the fruit of this or that plant. [Lashkhi, 1970; Писарницкий, 1966]. Thus, the higher alcohol with the smell and taste of honey - tyrosol is both in honey and in its alcoholic fermentation product, at the beginning of alcoholic fermentation its amount is 3 mg/dm<sup>3</sup>, after fermentation the amount of tyrosol in honey wine reaches 14 mg/dm<sup>3</sup>.

#### REFERENCES:

1. Alzahrani, H. A., Boukraa, L., Bellik, Y., Abdellah, F., Bakhotmah, B. A., Kolayli, S., & Sahin, H. (2012). Evaluation of the antioxidant activity of three varieties of honey from different botanical and geographical origins. *Global Journal of*

- Health Science, 4(6), 191. doi:10.5539/gjhs.v4n6p191
2. Barez, J. G., Garcia-Villanova, R. J., Garcia, S. E., Pal\_a, T. R., Param\_as, A. G., & S\_anchez, J. S. (2000). Geographical discrimination of honeys through the employment of sugar patterns and common chemical quality parameters. *European Food Research and Technology*, 210, 437–444. doi: 10.1007/s002170050578
3. Cotte, J. F., Casabianca, H., Giroud, B., Albert, M., Lheritier, J., & Grenier-Loustalot, M. F. (2004). Characterization of honey amino acid profiles using high-pressure liquid chromatography to control authenticity. *Analytical and Bioanalytical Chemistry*, 378(5), 1342–1350. doi:10.1007/s00216-003-2430-z
4. Cotte, J.F., Casabianca, H., Giroud, B. et al. Characterization of honey amino acid profiles using high-pressure liquid chromatography to control authenticity. *Anal Bioanal Chem* 378,1342–1350 (2004). <https://doi.org/10.1007/s00216-003-2430-z>
5. da Silva PM, Gauche C, Gonzaga LV, Costa AC, Fett R. Honey: Chemical composition, stability and authenticity. *Food Chem.* 2016 Apr 1;196:309-23. doi: 10.1016/j.foodchem.2015.09.051. Epub 2015 Sep 16. PMID: 26593496.
6. Erturk, O., Sahin, H., Kolayli, S., & C,ol Ayvaz, M. (2014). Antioxidant and antimicrobial activity of east black sea region honeys. *Turkish Journal of Biochemistry*, 39(1), 99–106. doi:10.5505/tjb.2014.77487
7. Krystyna Pyrzynska, Magdalena Biesaga, Analysis of phenolic acids and flavonoids in honey: *Trends in Analytical Chemistry*, Vol. 28, No. 7, 2009
8. Meo, S. A., Al-Asiri, S. A., Mahesar, A. L., & Ansari, M. J. (2017). Role of Honey in Modern Medicine. *Saudi Journal of Biological Sciences*, 24(5), 975–978.

9. Pereira V, Pontes M, Câmara JS, Marques JC. Simultaneous analysis of free amino acids and biogenic amines in honey and wine samples using in loop orthophthalaldehyde derivatization procedure. *J Chromatogr A*. 2008 May 2;1189(1-2):435-43. doi: 10.1016/j.chroma.2007.12.014. Epub 2007 Dec 15. PMID: 18164022.
10. Pereira AP, Dias T, Andrade J, Ramalhosa E, Estevinho LM. Mead production: selection and characterization assays of *Saccharomyces cerevisiae* strains. *Food Chem Toxicol*. 2009 Aug;47(8):2057-63. doi: 10.1016/j.fct.2009.05.028. Epub 2009 May 27. PMID: 19481129.
11. Rahman MM, Alam MN, Fatima N, Shahjalal HM, Gan SH, Khalil MI. Chemical composition and biological properties of aromatic compounds in honey: An overview. *J Food Biochem*. 2017;e12405.
12. S. G. Khafizova; L. V. Permyakova, V. A. Pomozova, 2013. Improving the technology of low-alcohol drinks based on honey, Beer and drinks 3 • 2013.
13. Syazana, M. S., Gan, S. H., Halim, A. S., Shah, N. S. M., & Sukari, A. (2012). Analysis of volatile compounds of Malaysian tualang (*Koompassia Excelsa*) honey using gas chromatography mass spectrometry. *African Journal of Traditional, Complementary and Alternative Medicines*, 10(2), 180–188.
14. [https://www.researchgate.net/profile/Jong-Whan\\_Rhim](https://www.researchgate.net/profile/Jong-Whan_Rhim)
15. Jong-Whan Rhim, Dong-Han Kim, Soon-Teck Jung, Production of Fermented Honey Wine, January 1997, *Korean Journal of Food Science and Technology* 29(2)
16. Хоситашвили М., Гоциридзе О., Гулиашвили М., Муджири Л., 1999. О содержании Тирозола в меде и алкогольных продуктах его переработки. *Georgian engineering news*; N 4 (8), с. 92.