Influence of Organochlorine Pesticides on Biochemical Transformations in the Process of Obtaining Apple Wine Materials

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Abstract. Today’s the problem of food security is the object of close attention of the world scientific community and international organizations. Food is the most important material basis for the existence of mankind and a necessary condition for the development of human civilization as a whole. Food safety is the part of the country’s national security; it ensures the life of the entire society and determines the economic, political and social independence of the state. In recent years, there has been a sharp deterioration in the quality of food around the world, which is primarily due to the chemization of agriculture in order to increase production and solve the problem of food shortages. The use of pesticides to control weeds, diseases and pests increases yields, but these substances accumulate in the soil, and through it in crops. However, as a result, pesticides are directly or indirectly introduced into agricultural products and from them into food products. Pesticides are xenobiotics that are foreign to living organisms and are not part of the natural biotic cycle. The consumption of pesticides with food harms human health. Pesticides also play a negative role in the technological processes of food production using microorganisms, for example, in wine-making, pesticides worsen the fermentation activity and the physiological state of yeast. The article presents the results of research on the effect of organochlorine pesticides on biochemical transformations in the process of obtaining apple wine materials.

1. Introduction

Ensuring food safety is the most important task of modern production. Despite the legal restrictions in Russia on the content of pesticides and other xenobiotics in food raw materials and products, the level of their contamination with pesticides remains quite high [1-3].

Taking into account the increasing danger to human health of consuming food containing xenobiotics, the international standard ISO 22000 has been developed. It contains requirements for the functioning of an effective food safety management system based on the principles of the HACCP
(Hazard Analysis and Critical Control Point) system and on the implementation of pre-conditions necessary for the production of safe food [4-7].

Pesticides (herbicides, insecticides, fungicides, nematicides, fertilizers and soil amendments) enter food products through direct processing of agricultural objects in order to destroy weeds, diseases and pests, or through contaminated water and soil. A distinctive feature of many pesticides is their high resistance to environmental factors and their ability to persist in soil for 10 or more years. Also, pesticides accumulate in soil and plants, which leads that in many cases their content in food is dangerous for human health. The most dangerous are persistent and toxic pesticides, first of all, organochlorine compounds [8-11].

To date, the pesticide from the group of organochlorine compounds hexachlorocyclohexane (HCH) is widely used in agriculture. The isomers of HCH are poisonous and persistent organic pollutants, the most toxic is γ-HCH, which has the technical name lindane and is used as an insecticide. HCH has pronounced cumulative properties - it accumulates in animal organisms, in plants (HCH penetrates through leaves and roots into fruits, tubers and other organs, thereby making them phytotoxic). Since 2009 lindane is banned from production in 50 developed countries, but is widely used in countries with agricultural economies, and seventeen countries, including the United States and Canada, have allowed themselves limited agricultural use [11-14].

The widespread use of pesticides in plant raw materials and their high toxicity led to the development of methods for detoxifying raw materials and finished products. One of the most promising methods is biological, based on the use of microorganisms. All types of microorganisms, including industrial yeast of the genus Saccharomyces, used in fermentation processes of grape or apple musts to wines, have the ability to biotransformation of pesticides. Yeast of the genus Saccharomyces consume organochlorine pesticides as a source of carbohydrate nutrition and reduce their concentration level, depending on the initial content, by 60-100 %. However, pesticides negatively affect the fermentation activity and the physiological state of yeast [15, 16].

2. Experimental section
The main purpose of the research is to study the influence of organochlorine pesticides on the formation of organic acids and amino acids in the process of obtaining apple wine materials, as well as on changes in the qualitative and quantitative composition of aroma-forming compounds. To achieve this goal, model experiments were conducted.

Experimental samples of apple must containing HCH in an amount corresponding to the maximum permissible concentration - 0.4 mg/dm3 and in an amount exceeding the maximum permissible concentration by 10 times - 4 mg/dm3 were prepared. As a control sample was used apple must, which does not contain a toxin, and the resulting apple wine material.

Pure cultures of Saccharomyces cerevisiae yeast were used for fermentation. The yeast immobilization process was performed on oak chips and on polyethylene rings in accordance with the recommendations received earlier [17, 18]. Active biomass of yeast cultures in the amount of 2% of must volume or 10 million cells in 1 cm3 of must was used for must seeding.

The content of organic acids was determined by high-performance liquid chromatography (HPLC) using a liquid chromatograph "Stayer" with a spectrophotometric detector. Column – Kromasil C18 HPLC, 5 μm particle size, pore size 100 Å, L × I.D. 250 mm × 4.6 mm. The volume of the injected sample was 20 μl.

Amino acids were determined using the Hewlett Packard AminoQuant M Ser Amino Acid Analyzer the Hitachi 835 High-Speed Amino Acid Analyzer.

The content of aroma-forming compounds and glycerol was determined using a gas-liquid chromatograph Hewlett Packard with a Multichrome data collection board (GLC/FID-analysis and GLC/MS-analysis).
3. Results section

The influence of HCH on changes in the qualitative and quantitative composition of organic acids was studied. The obtained results are shown in table 1 and figure 1.

Table 1. The influence of HCH on the content of organic acids in the apple wine materials, g/dm$^3$

| Organic acid       | Apple wine materials |
|--------------------|----------------------|
|                    | Apple must | Apple wine material (control) | Apple wine material (HCH 0.4 mg/dm$^3$) | Apple wine material (HCH 4 mg/dm$^3$) |
| Malic acid         | 5.76       | 5.45                       | 5.56                                    | 5.48                               |
| Lactic acid        | -          | 0.20                       | 0.07                                    | 0.10                               |
| Citric acid        | 0.10       | trace amounts              | trace amounts                           | trace amounts                       |
| Shikimic acid      | 0.02       | 0.03                       | 0.03                                    | 0.03                               |
| Acetic acid        | 0.07       | 0.43                       | 0.62                                    | 0.78                               |
| Quinic acid        | 0.86       | 0.1                        | 1.10                                    | 1.02                               |
| Fumaric acid       | 0.01       | 0.10                       | 0.06                                    | 0.08                               |
| Sum                | 6.84       | 6.31                       | 7.44                                    | 8.48                               |

Figure 3. The influence of HCH on the content of organic acids in the apple wine materials, g/dm$^3$.

The results of studies of the qualitative and quantitative composition of free amino acids in samples of apple wine materials are presented in table 2.
The results of studies of the qualitative and quantitative composition of amino acids in polypeptide chains in samples of apple wine materials are presented in table 3.

**Table 2. The influence of HCH on the content of free amino acids in the apple wine materials, mg/dm$^3$**

| Amino acid  | Apple must | Apple wine material (control) | Apple wine material (HCH 0.4 mg/dm$^3$) | Apple wine material (HCH 4 mg/dm$^3$) |
|-------------|------------|-------------------------------|----------------------------------------|-------------------------------------|
| Aspartic acid | 5.72       | -                             | -                                      | -                                   |
| Serine      | 7.06       | -                             | -                                      | -                                   |
| Glutamic acid | 18.46     | 0.51                          | 1.25                                   | 1.31                                |
| Proline     | 1.91       | -                             | -                                      | -                                   |
| Alanin      | 0.761      | 0.157                         | 0.11                                   | 0.162                               |
| Cystine     | 5.86       | 5.41                          | 5.09                                   | 5.59                                |
| Leucine     | 8.03       | 2.45                          | 1.12                                   | 2.58                                |
| Tyrosine    | -          | -                             | 0.97                                   | 0.94                                |
| Phenylalanine | -         | -                             | -                                      | 0.103                               |
| Lysine      | -          | 0.7                           | 1.01                                   | 0.98                                |
| Histidine   | 2.42       | 0.259                         | 2.13                                   | 2.13                                |
| Sum         | 55.55      | 8.17                          | 11.94                                  | 9.87                                |

**Table 3. The influence of HCH on the content of amino acids in polypeptide chains in the apple wine materials, mg/dm$^3$**

| Amino acid  | Apple must | Apple wine material (control) | Apple wine material (HCH 0.4 mg/dm$^3$) | Apple wine material (HCH 4 mg/dm$^3$) |
|-------------|------------|-------------------------------|----------------------------------------|-------------------------------------|
| Aspartic acid | 121.02     | 16.32                         | 38.54                                  | 65.54                               |
| Threonine   | -          | 6.88                          | 1.68                                   | 36.34                               |
| Serine      | 10.24      | 7.88                          | 2.32                                   | 41.88                                |
| Glutamic acid | 43.92     | 31.82                         | 4.60                                   | 89.94                                |
| Proline     | 12.04      | 17.28                         | 1.20                                   | 11.29                                |
| Glycine     | 4.64       | 8.88                          | 2.48                                   | 51.93                                |
| Alanine     | 11.34      | 10.12                         | 1.60                                   | 62.01                                |
| Cystine     | 2.68       | 3.14                          | 1.48                                   | 20.41                                |
| Valine      | 4.72       | 10.72                         | 1.82                                   | 37.32                                |
| Methionine  | 2.78       | 4.34                          | 1.90                                   | 18.71                                |
| Isoleucine  | 3.18       | 8.32                          | 1.00                                   | 31.82                                |
| Leucine     | 4.60       | 13.58                         | 1.10                                   | 29.25                                |
| Tyrosine    | 2.82       | 3.74                          | 1.66                                   | 40.00                                |
| Phenylalanine | 12.76     | 8.18                          | 0.58                                   | 20.75                                |
| Lysine      | 2.18       | 3.72                          | 0.80                                   | 32.85                                |
| Histidine   | 1.92       | 2.78                          | 0.48                                   | 21.63                                |
| Arginine    | 1.158      | 4.08                          | 0.24                                   | 54.80                                |
| Sum         | 237.00     | 161.00                        | 29.00                                  | 696.70                               |
The results of the study of the influence of HCH on the change in the qualitative and quantitative composition of aroma-forming compounds are presented in table 4.

**Table 4.** The influence of HCH on the content of aroma-forming compounds in the apple wine materials, mg/dm³

| Aroma-forming compound | Apple must | Apple wine material (control) | Apple wine material (HCH 0.4 mg/dm³) | Apple wine material (HCH 4 mg/dm³) |
|------------------------|------------|-------------------------------|-------------------------------------|----------------------------------|
| Acetaldehyde           | 10.5       | 18.7                          | 2.6                                 | 34.7                             |
| Methyl acetate         | 0.6        | 10.1                          | 4.5                                 | 11.9                             |
| Ethyl acetate          | 4.8        | 6.0                           | 6.8                                 | 12.4                             |
| Methanol               | 4.3        | 25.2                          | 124.0                               | 151.0                            |
| Butanol-2              | -          | -                             | -                                   | -                                |
| n-Propanol             | -          | 3.4                           | 2.8                                 | 3.1                              |
| Isobutanol             | 9.9        | 20.1                          | 7.9                                 | 31.0                             |
| Butanol-1              | -          | -                             | -                                   | -                                |
| Isoamyl alcohol        | 14.8       | 34.0                          | 43.5                                | 53.1                             |
| Ethyllactate           | 4.1        | 21.0                          | 32.2                                | 18.0                             |
| Butanediol-2,3         | 44.9       | 81.2                          | 80.7                                | 83.5                             |
| Butanediol-1,4         | 20.2       | 25.2                          | 3.5                                 | 58.9                             |
| Phenylethyl alcohol    | 18.3       | 63.9                          | 84.1                                | 96.9                             |

4. Discussion section
As shown in table 1 and figure 1, during fermentation of apple must containing HCH, there is an increase in the total amount of organic acids in all samples of wine materials, mainly due to acetic acid, which negatively affects the quality of the resulting wine materials. There was also an increase in the content of lactic and quinic acids. Given that quinic acid is formed during the glycolytic decomposition of sugars from phosphoenolpyruvic acid, both latter acids can be considered as secondary metabolites formed from sugars. In all the analyzed samples of wine materials, there was a slight decrease in the content of malic acid. An increase in the total content of organic acids above the optimal level negatively affects the overall organoleptic evaluation of the obtained wine materials.

As shown in table 2, the qualitative and quantitative composition of amino acids in all samples of apple wine materials changes in comparison with the original apple must. Eight amino acids have been identified in apple must. The largest amount contained glutamic acid (44.3 % of the total amount of amino acids), the smallest – alanine (1.2%), and tyrosine, phenylalanine and lysine were found in trace amounts.

In the process of fermentation, the yeast is actively assimilated aspartic acid, serine and proline, the total content of amino decreased compared to the control. Data from table 3 indicate that 16 amino acids in polypeptide chains were found in apple must and in the wine materials obtained from it, with aspartic and glutamic acids in the largest amounts. During fermentation of apple must, the content of amino acids in the wine material (HCH 0.4 mg/dm³) significantly decreased, the concentration of amino acids was 5.5 times less than in the apple must without HCH. When the experimental sample contains 4 mg/dm³ of HCH, the process of destruction of yeast cells occurs intensively, leading to an increase in the content of amino acids.

As can be seen from the data presented in table 4, the content of all aroma-forming compounds identified in apple must increased in all experimental samples of wine materials.
Thus, the experimental samples had a higher content of esters, acetaldehyde, and higher alcohols: isobutanol and isoamyl alcohol compared to the control. During fermentation in the control apple must there was an accumulation of acetaldehyde, methanol, isobutanol, isoamyl alcohol, butanediol and phenylethyl alcohol.

In a sample containing 4 mg/dm³ of HCH in the apple must, an increase in the concentration of methanol, acetaldehyde, methyl acetate, ethyl acetate, isobutanol and isoamyl alcohol was recorded, which negatively affects the quality of the product.

In the samples containing HCH, secondary metabolites - volatile acids and glycerol - accumulated more intensively, as well as the concentration of higher alcohols increased. Excessive accumulation of higher alcohols leads to a deterioration in the organoleptic characteristics of the finished product.

5. Conclusions
The influence of organochlorine pesticides on biochemical transformations in the process of obtaining apple wine materials has been studied and their role in enhancing the formation of secondary fermentation products – aldehydes, higher alcohols and volatile acids, which negatively affect the quality of products, has been established. Thus, the research indicates significant changes in the composition of apple wine materials under the influence of pesticides.

6. References
[1] Harsimran K G and Harsh G 2014 Pesticides - Toxic Effects, (Berlin: ResearchGate)
[2] Coppola L, Comitini F, Casucci C, Milanovic V, Monaci E, Marozinzi M, Taccari M, Ciani M, Viscetti C 2011 New Biotechnology 29 99-106
[3] Abdullah N Z, Ishaka A, Samsuddin N, Mohd Rus R, Mohamed A H 2011 IIUM Research, Invention and Innovation Exhibition 223
[4] Agrawal A, Sharma B 2010 Int. J. of Biological and Medical Research 29 99-106
[5] Armstrong J L, Fenske R A, Yost M G, Galvin K, Tchong-French M, Yu J 2013 Atmospheric Environment 66 145-15
[6] Cothran R D, Brown J M, Relyea R A 2013 Evolutionary Applications 6 832-841
[7] Anand M, Taneja A 2020 Environ. Res. 182 109106
[8] Zhang D, Liang P, Yu Z, Xia J, Jin S 2020 J. Haz. Mat. 382 121023
[9] ISO 22000:2005 Food safety management systems. Requirements for any organization in the food chain
[10] Bokulich N A, Thorngated J H, Richardsone P M, Mills D A 2013 Proc. Natl. Acad. Sci. U.S.A. 111 E139–E148
[11] Jolly N P, Varela C, Pretorius I S 2014 FEMS Yeast Res. 14 215–237
[12] Čuš F, Gregorčič A, Baša Cesnik H, Bolta S 2010 Food Control 21(2) 150-154
[13] Bityutskii N, Pavlovic J, Yakkonen K, Maksimovi V, Nikolic M 2014 Plant Physiol. Biochem. 74 205–211
[14] Cornelis J T, Delvaux B, Georg R B, Lucas Y, Ranger J, Opfergelt S 2011 Biogeoscience 8 89–112
[15] Deshmukh R, Vivancos J, Guerin V, Sonah H, Labbe´ C, Belzile F 2013 Plant Mol. Biol. 83 303–315
[16] Mas A, Guillamon G M, Torija M J, Beltran G, Cerezo A B, Troncoso A M and Garcia-Parrilla M C 2014 BioMed Research International Article ID 898045
[17] Panasyuk A L, Kuzmina E I, Kharlamova L N, Babaeva M V and Romanova I P 2019 IOP Conference Series: Materials Science and Engineering 582 012011
[18] Oganesyants L A 1998 Scientific justification and development of technology for wine production using oak wood Abstract. Diss. d. t. n. 68