Toxins and Mechanisms of Their Interaction in Fruit Wines

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Abstract*: The purpose of the work was to ensure the environmental safety of fruit wines based on studying the content of secondary metabolites of mold fungi – mycotoxins – and their properties in them. The study objects included different varieties of apples that were damaged in a different way. The whole apples that were not damaged by pests and diseases were used as a reference group. To define the content of patulin and aflatoxins A and G, thin layer chromatography and high-performance liquid chromatography were used. In order to define the mass concentrations of amino acids, the method of liquid ion-exchange eluent chromatography was used. The analysis of wine materials produced from apples that had various types of damage has showed that they contained mycotoxins, namely, aflatoxins A and G and patulin. It has been determined that the degree of contamination of the apples surface with mold fungi depends on the type of the fruit damage. The content of toxins increases in the following sequence: mechanically damaged apples < pest damaged apples < apples damaged by diseases. All objects were characterized by a considerable content of patulin. An attempt has been made to define possible areas for the patulin synthesis with mold fungi. The impact of mold fungi on the composition of amino acids in fruit materials has been studied. The difference in the concentration of amino acids in low alcoholic and highly alcoholic environments has been revealed. It has been defined that the reason for obtaining fermented juices with a depleted amino acid composition is the use of the fruits damaged with rot. The increase in the content of certain amino acids in fermented alcoholized juices indicates the inhibitory effect of the alcoholic medium on the metabolic processes of mold fungi and a decrease in their enzymatic activity. The mechanism of the interaction between patulin and amino acids of fruit materials has been explained.

Index Terms*: aflatoxin, amino acids, fruit wines, mechanisms, patulin.

I. INTRODUCTION

The competitiveness of wine products in the current market conditions determines the further development of the wine industry in Russia. At the same time, the environmental safety is one of the main criteria for assessing the quality of wine. The presence of mold fungi and their metabolites, highly toxic compounds – mycotoxins, is the least studied factor that considerably affects the quality and safety of raw materials and finished products. There are more than 250 species of microorganisms that synthesize over 100 mycotoxins. Mycotoxins found in agricultural raw materials, including those intended for winemaking, have been studied little or have not been studied at all. References contain certain data on aflatoxin and patulin found in fruits, vegetables, and juices produced of them [1]-[5]. According to the studies of L.V. Donchenko and V.D. Nadykta [6], [7], up to 136 mg of patulin per 1 kg of fruit were found in the flesh of apples affected by rot. It is necessary to note the fact that Sanitary Rules and Norms (SanRaN) 2.3.2.107-01 regulate only the maximum amount of patulin in fruits and juices and do not normalize the content of aflatoxin A and G, despite their high toxicity, strong mutagenic, hepatogenic, and teratogenic immunodepressive action. It is known that the interaction of aflatoxin and nucleophilic sites of DNA, RNA and proteins determines its toxic effect [8]. In Russia, ochratoxin A was identified for the first time in the wine materials obtained from grapes of the Anapa and Taman zones by T.I. Guguchkina, I.N. Ageeva, and Yu.F. Yakuba [9]. Its amount varied from 0.22 to 8.5 $\mu g/dm^3$ and depended on the rot damage of the processed grapes, as well as on their variety.

II. LITERATURE REVIEW

For the first time, the issue on the contamination of products with ochratoxins produced by such species of fungi as Aspergilus and Penicilium, in particular, A. ochraclus P. ciridicatum and others was mentioned in works in the 1960s. According to the materials of the General Assembly of the International Organization of Vine and Wine (AIOVW) (Paris, 2000), ochratoxin was found in wines produced in Italy, Australia, Switzerland, and Germany [16], [17]. At the same time, the International Agency for Research on Cancer classified ochratoxin as a potentially carcinogenic substance [15]-[17]. According to the analysis of references, toxins of fruit wines have almost not been studied. Meanwhile, in order to obtain fermented and fermented-alcoholized juices in the production of fruit wines, the fruits damaged by pests and diseases, as well as the so-called drop are used. This fact suggests toxins in fruit wines. Thus, the studies aimed at researching toxins of fruits and products made

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of them, in particular fruit wine materials, are relevant and practically significant [18]-[20]. According to the references, there are several ways for the biosynthesis of mycotoxins [18], [19]. The main one is polyketide [20], [21] that results in the formation of most mycotoxins. It is based on the linear condensation of acetyl C_oA and three or more molecules of malonyl C_oA, and simultaneous decarboxylation, while there is no indispensable reduction of intermediate β-dicarbonyl compounds [20], [21]. References do not contain any information about possible ways and mechanisms of the patulin formation, as well as its importance in forming quality indicators of wine. Due to this, based on the references and their own experimental data, the authors made an attempt to consider possible directions of the patulin biosynthesis.

The goal of the work is to ensure the environmental safety of fruit wines, based on studying the content of secondary metabolites of mold fungi – mycotoxins, and their properties.

III. OBJECTS AND METHODS

The study objects included different varieties of apples that were damaged in a different way. The whole apples that were not damaged by pests and diseases were used as a reference group.

IV. PROPOSED METHODOLOGY

In order to define the content of patulin and aflatoxins A and G, thin-layer chromatography according to GOST R 51440-99 (ISO 8128.2-93) and high-performance liquid chromatography method according to GOST R 51433-99 were used. In order to define the mass concentrations of amino acids, the method of liquid ion-exchange eluent chromatography was used. It involved the use of the AAA-881 automatic amino acid analyzer (Czechoslovakia) in the mode of analyzing hydrolysers by applying sodium nitrate buffers with pH values of 3.25; 4.25; 5.28 [10].

A. Algorithm

During the study, the content of toxins in the juices produced from the whole (reference group) and damaged apples (experimental group) was defined. The toxins found in the damaged apples necessitate determining the mechanism of their formation in fruit wines. In terms of chemistry, patulin (clavatin, clavicin, claviformin, mycoin C, mucoin, penicidin) is 4-hydroxyfuropyran that has two functional groups:

1) Hydroxyl – OH group that by analogy with phenols has acidic but more pronounced properties, as a result of the *p*-conjugation of the electron pair of the oxygen atom of the pyran ring,

2) The carbonyl group that has a negative mesomeric effect, contributing to an increase in both a partially negative charge on the oxygen atom, and a partially positive charge on the carbon atom, which makes it possible for patulin and amino acids to interact.

Besides, the qualitative and quantitative composition of amino acids of fruit materials was defined.

B. Flow Chart

The study was carried out according to the below scheme (Table I).

Table I. Flow Chart

Group	Description
Stage 1 of the study (defining mycotoxins in apple juices)	
1 reference	Juice of the undamaged apples
2 experimental	Juice of the mechanically damaged apples
3 experimental	Juice of the pest damaged apples
4 experimental	Juice of the apples damaged by fungi
Stage 2 of the study (defining the amino acid composition of juices):	
Fermented alcoholized juices	
1	made of the whole apples
2	made of the damaged apples
Fermented juices	
3	made of the whole apples
4	made of the damaged apples

V. RESULTS

The results of the first stage presented in Table II show the qualitative and quantitative content of mycotoxins in the juices made of apples with different types of damage.

Table II. Content of Toxins in the Juice Depending on the Type of Apples Damage

Mass concentration of toxins mg/kg	Group			
	1 (reference)	2 (experimental)	3 (experimental)	4 (experimental)
Patulin	no	0.0018	0.0034	0.085
Aflatoxin A	no	0.014	0.023	0.078
Aflatoxin G	no	0.008	0.0016	0.026

The obtained results confirm the complete absence of toxins in the juice produced from the undamaged apples (reference). The juice produced of apples with various types of damage contained aflatoxin G, aflatoxin A, and patulin. Their concentration increased in the following order: mechanically damaged apples > apples damaged by pests > apples damaged by mold fungi. At the same time, the content of patulin was the highest in all samples. Its concentration in the juice produced of the apples damaged by mold fungi was 0.085 mg/kg, while the norm was not higher than 0.05 mg/kg (SanRan 2.3.2. 1078-01). It is known that aflatoxins are the waste products of the Aspergillus flavus fungi [11], [12], while patulin – of the Penicillium expansum fungi [5], [13], [14]. Mycotoxins are synthesized from a rather limited number of products of the main metabolism, such as acetate, mevalonate, and certain amino acids. The use of the nuclear magnetic resonance method confirmed the biosynthesis of mycotoxins from chemically simple intermediate products, namely, organic acids and amino acids during the condensation, redox, alkylation, and cyclization reactions [15]-[17].

Two pairs of paired electrons in oxygen atoms determine the main properties, which contributes to reducing the acidity of solutions containing patulin.

Table III shows the amino acid composition of the fermented and fermented- alcoholized juices made during stage two of the study from the whole apples and those damaged by mold fungi.

Table III. Qualitative and Quantitative Composition of Amino Acids of Fruit Materials, mg/dm^3

Amino acid	Fermented-alcoholized juices		Fermented juices	
	made of the whole apples	made of the damaged apples	made of the whole apples	made of the damaged apples
Alanine	66.2	92.4	146.8	64.8
Aminobutyric	16.5	10.5	23.8	8.8
Asparagin	43.6	15.6	112.8	56.4
Valin	8.4	1.2	11.7	6.4
Glutamine	132.4	104.8	144.2	108.8
Glycine	6.2	12.0	10.6	8.8
Histidine	6.0	8.2	8.8	6.8
Isoleucine	16.4	14.8	32.8	20.2
Leucine	12.0	12.9	36.0	24.5
Lysine	12.0	10.6	24.2	14.4
Methionine	6.6	3.6	12.9	8.0
Proline	226.8	140.6	280.6	240.8
Serine	18.8	6.4	60.2	15.0
Tyrosine	19.6	24.8	44.2	24.0
Threonine	8.2	2.8	54.2	14.7
Phenylalanine	2.0	3.8	4.0	1.6
Cystine + cysteine	34.6	46.8	50.0	24.2

The analysis of the effect of mold fungi on the qualitative and quantitative composition of the amino acids in the juices showed that the concentration of all identified amino acids in the fermented (low alcoholized) juices produced from damaged apples was lower than in the juices produced from the whole apples. Namely, the content of threonine, aminobutyric acid decreased 3 times, and the content of aspargin, alanine, valine, phenylalanine, tyrosine, as well as the amount of cysteine and cystine – more than twice. The concentration of the remaining amino acids decreased by 10 – 40%. The analysis of the fermented-alcoholized juices (highly alcoholic) produced from the damaged fruits showed that, along with a considerable decrease in the content of some amino acids, such as, valine – eight times, asparagine – three times, proline and methionine – two times, aminobutyric acid – 1.5 times, there was a considerable increase in the content of other amino acids, namely, phenylalanine – two times, alanine – 1.4 times, glycine, as well as tyrosine – by 20%, and the total of cystine and cysteine – by 20 – 30%. The obtained results suggest that the use of the fruits affected by rot contributes to producing low alcoholic fermented juices with a depleted amino acid composition associated with

– Using of amino acids by mold fungi as sources of carbon and nitrogen,

– Transformations of amino acids under the action of oxidative enzymes of mold fungi, and

– Intensive consumption of amino acids by yeast during the fermentation of juice on the background patulin.

The increase in the content of certain amino acids in highly alcoholic fermented-alcoholized juices produced from the fruits damaged by mold fungi indicates a decrease in the effect of mycotoxins on the accumulation of amino acids if the alcohol capacity of the medium increases because of the inhibition of the metabolic processes of mold fungi and a decrease in their enzymatic activity, which affects the toxins biosynthesis processes. The increase in the electron density on the nitrogen of the NH_2 amino group that is enhanced by the positive inductive effect of the methyl group causes threonine to react with patulin. The scheme of interaction between patulin and alanine, one of the most active amino acids of fruit wines, shows a possible process causing a change in the chemical composition and, consequently, the quality of wine as a result of the vital activity of mold fungi.

At the same time, the serine concentration possibly decreases according to the electron-withdrawing mechanism.

VI. CONCLUSION

Thus, the studies have shown that the fruit materials made of the apples with various types of damage contain aflatoxins A and G and patulin, and their highest content was revealed when the fruits had been damaged by diseases. The results obtained indicate that the fruits damaged by rot contribute to the production of low alcoholic fermented juices with a depleted amino acid composition.

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Therefore, in order to improve the safety of fruit drinks, it is necessary to take measures on preventing and removing toxins from them, which is an important stage for the further study.

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