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EFFECT OF WATER ACTIVITY, pH AND TEMPERATURE ON CONTAMINATION LEVEL OF DRIED VINE FRUITE BY FILAMENTOUS FUNGI DURING STORAGE

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Growth of filamentous fungi in grape processing products depends on physical, chemical and biological factors. Our researches revealed high contamination level of dried vine fruit samples by filamentous fungi at high values of water activity. Definite correlation between values of acidity (pH) and sporulation level of dried vine fruit by filamentous fungi is revealed as well. The effect of temperature conditions of storage on mycobiota and contamination level of Armenian dried vine fruit by microscopic filamentous fungi is also studied. Storage of dried vine fruit in temperature lower then 4°*C* conditions promotes reduction of its sporulation degree by filamentous fungi.

Keywords: Filamentous fungi, water activity, acidity, sporulation degree, dried vine fruit

Introduction. The growth of fungi and mycotoxin synthesis is conditioned by variety of biotic and abiotic parameters. Water activity (aw) is the most important factor influencing on sporulation and growth of fungi and mycotoxin production. Temperature has an important role on these processes as well, but its effect on growth of fungi is always studied with aw [1, 2]. On the other hand, it is too important to study the effect of each parameter on growth of fungi separately as to prevent food contamination by filamentous fungi [3].

According to [4], the minimum value of aw necessary for growth of filamentous fungi is in range of 0.65–0.77. The optimum value of aw for growth of *Aspergillus carbonarius* is in the range of 0.93–0.9 [5, 6] and for *A. niger* aw is 0.97 [7, 8]. Species of filamentous fungi named above are dominant mycobiota of dried vine fruit. The value of aw for fungi growth varies depending on temperature conditions, type of substrate and the region, in which fungi were detected.

Among ecological factors environment temperature is the key parameter influencing on growth and evolution of filamentous fungi. The species of genus *Aspergillus* have a large range of temperature growth and the maximum temperature is 45°*C*. The minimum temperature for *A. niger* growth is 6–8°*C*, the maximum

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temperature is 45–47°*C* and the optimum temperature is 35–37°*C* [9]. The optimum temperature for *A. carbonarius* growth is 30–35°*C* [10]. The temperature value for *A. ochraceus* growth is at the range of 10–40°*C* [11]. The species *P. verrucosum* can grow and synthesize ochratoxin A (OTA) at lower temperature conditions, and the growth range is 10–31°*C*. This explains the fact that the species of genus *Aspergillu*s dominated in countries with warm and humid climate [12]. These are mainly countries in Africa, South Asia and South America. In Northern Europe, where the cool temperature climate predominates, the high contamination level of dried food is associated with species of *Penicillium* genera: *P. verrucosum* and *P. nordicum*.

Materials and Methods. For isolation of filamentous fungi CYA (Chapek-yeast agar medium), GYA (Glucose-yeast agar medium), MEA (Malt-extract agar medium), CDA (Chapek-dox agar medium) produced by "HiMedia Ltd.", were used. The plates were incubated at 28°*C* for 7 days (NF ISO 7954-88). After incubation the colony forming unit (CFU) was accounted according to NF ISO 7698-91. The growing fungi were identified morphologically based on macro- and microscopic characteristics using the following manual [13–17].

Determination of aw of dried vine fruit samples was spent with AquaLab (Decagon Devices, Pullman, WA, USA). Definition of pH was carried out with pH-meter (Oakton, USA).

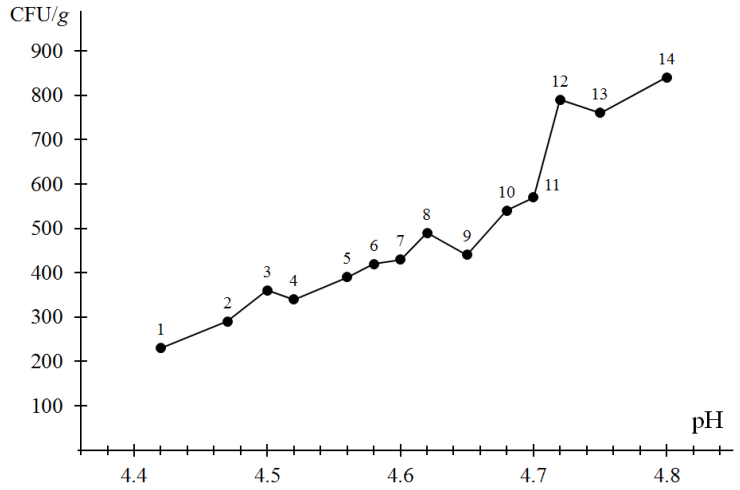
Results and Discussion. Water activity is the most important factor determining growth, evolution and adhesion degree of moulds on nutrient substrates, including dried vine fruit [18]. The results of researches of the effect of water activity on contamination level of dried vine fruit samples by filamentous fungi are given in Fig. 1. Definite correlation between values of aw and sporulation level of dried vine fruit by filamentous fungi is shown in our researches. Dependence between these parameters is absent in some samples with low value of water activity. But from aw=0.56 value an exact correlation between aw and contamination level of dried vine fruit by filamentous fungi is registered.

The acidity (pH) of nutrient medium and substrate essentially influences on growth of fungi from *Aspergillus* and *Penicillium* genera. According to [19], the optimum range of pH value necessary for active growth of *A. ochraceus* is of 3–10, for fungi from section *Aspergillus Nigri* the optimum pH value is 4.0–6.5. *A. carbonarius* species is able to grow and produce mycotoxins at more wide range of pH value: from 2 to 10. In studied samples the value of pH varies from 4.45 to 4.85 (Fig. 2). Such medium is optimally favorable for growth of micromycetes. The highest sporulation level of dried vine fruit is observed at pH value of 4.75–4.85. This pH value is typical for Armenian dried vine fruit as the glucose contents is high in raw grape. Acidity in grape berry is mainly determined with high contents of sugar in it.

The sugar content in varieties of seedless grapes intended for drying should not be less than 23–25%, and for seeded varieties the sugar content should not be less than 22–23% [20].

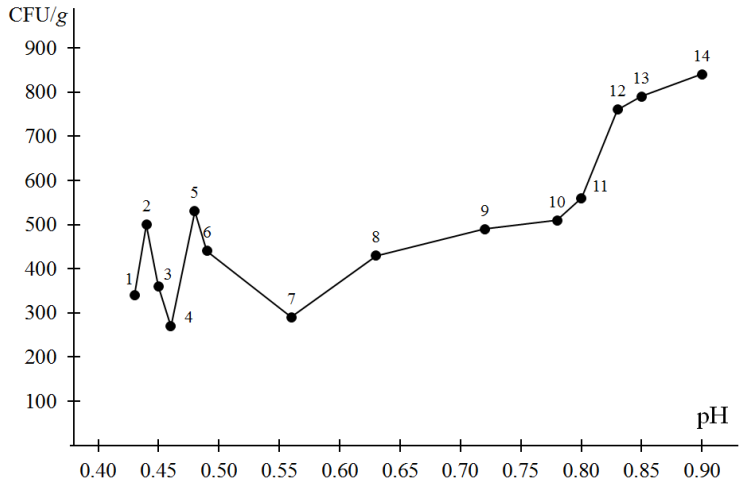
One of the goals of these researches is studying the influence of storage conditions on mycobiota of local produced dried vine fruit. The influence of temperature conditions on mycobiota and contamination level of local produced

dried vine fruit by filamentous fungi is studied. Repeated analyses of 13 samples of Armenian black dried vine fruit were carried out after six months storage at temperature lower then 4°*C* for detecting possible changes in mycobiota contaminating them. Raisin and sultana varieties of dried vine fruit were analyzed. Raisins are prepared from grapes that possess seeds, which may or may not be removed in processing. Sultanas are prepared from grapes that are naturally seedless or almost seedless (see Table).



	1	2	3	4	5	6	7	8	9	10	11	12	13	14
CFU/*g*	340	500	360	270	530	440	290	430	490	510	560	760	790	840
pH	0.43	0.44	0.45	0.46	0.48	0.49	0.56	0.62	0.72	0.78	0.80	0.83	0.85	0.90

Fig. 1. Correlation between aw and contamination level of dried vine fruit by micromycetes.



	1	2	3	4	5	6	7	8	9	10	11	12	13	14
CFU/*g*	230	290	360	340	390	420	430	490	440	540	570	790	760	840
pH	4.42	4.47	4.50	4.52	4.56	4.58	4.60	4.62	4.65	4.68	4.70	4.72	4.75	4.80

Fig. 2. Correlation between pH and contamination level of dried vine fruit by micromycetes.

The results of mycological analyses of experimental samples have shown that there is no essential change in compounds of species after six months storage at low temperature of black type of dried vine fruit. Domination of species from *Aspergillus* genera is observed in analyzed samples. In Table you can see that species from *Aspergillus* section *Nigri* are resistant enough against low temperature conditions storage of dried vine fruit during six months. In some samples of dried vine fruit *A. foetidus*, *A. niger* and *A. aculeatus* species were detected, which had not been revealed in samples analyzed immediately after drying.

Results of repeated analyses of Armenian black dried vine fruit after 6 months storage at low temperature

Place and date of sampling		Dominant mycobiota before storage	Dominant mycobiota after storage
Raisin	market 14.11.2008	*M. mucedo*, *A. carbonarius*, *A. niger*, *A. foetidus*	*A. niger*, *A. aculeatus*, *A. foetidus*, *A. carbonarius*
	market 07.12.2009	*M. mucedo*, *A. flavus*, *A. niger*, *P. rubrum*, *P. griseofulvum*	*A. foetidus*, *A. niger*, *A. flavus*, *P. rubrum*
	market 18.03.2009	*M. mucedo*, *A. carbonarius*, *A. aculeatus*, *P. lanosum*, *P. diversum*, *A. alternata*	*A. carbonarius*, *A. aculeatus*, *A. niger*,
	market 16.10.2008	*A. niger*, *A. carbonarius*, *A. foetidus*, *P. lanosum*	*A. foetidus*, *A. niger*, *A. carbonarius*, *P. lanosum*
	market 07.12.2009	*A. carbonarius*, *P. variabile*, *P. diversum*, *A. alternata*	*A. carbonarius*, *P. variabile*
	manufactur 26.12.2009	*M. racemosus*, *A. niger*, *P. cyclopium*	*M. racemosus*, *A. niger*, *P. cyclopium*, *P. rubrum*
	manufactur 26.12.2009	*M. mucedo*, *A. flavus*, *A. niger*, *P. griseofulvum*	*M. mucedo*, *A. niger*, *P. griseofulvum*
	manufactur 23.10.2010	*A. ochraceus*, *A. niger*, *P. chrysogenum*	*A. ochraceus*, *A. niger*, *P. chrysogenum*
Sultana	manufactur 26.10.2009	*A. niger*, *A. flavus*, *A. carbonarius*	*A. niger*, *A. flavus*, *A. carbonarius*
	market 04.02.2010	*M. racemosus*, *A. niger*, *A. flavus*, *A. carbonarius*, *P. velutinum*	*A. niger*, *A. flavus*, *A. carbonarius*
	manufactur 23.10.2010	*M. racemosus*, *A. niger*, *P. variabile*	*M. racemosus*, *A. niger*, *A. foetidus*, *P. variabile*
	manufactur 23.10.2010	*M. mucedo*, *A. foetidus*, *A. fumigatus*	*A. foetidus*, *A. niger*, *A. alternata*
	market 09.03.2008	*M. racemosus*, *A. niger*, *A. flavus*, *A. carbonarius*, *P. chrysogenum*, *P. brevicompactum*, *P. velutinum*	*M. racemosus*, *A. niger*, *A. carbonarius*, *A. aculeatus*, *A. flavus*, *A. foetidus*

Some species from *Penicillium* genera: *P. chrysogenum*, *P. griseofulvum* and *P. variabile* have also shown resistance against low temperature storage. These species from genus *Penicillium* are coremic fungi. The aggregation of conidiophores in coremia form increases their vitality. Species from *Mucor* genera occur rarely after six months storage.

Storage of dried vine fruit in low temperature condition promotes some reduction of its contamination level by filamentous fungi (Fig. 3).

The low value of CFU/*g* (colony forming unit) of samples after six months storage is explained by filamentous fungi growth inhibition at temperatures lower then 4°*C*. The results of this study could be used as a scientific basis for determining of storage conditions and shelf life of dried vine fruit.

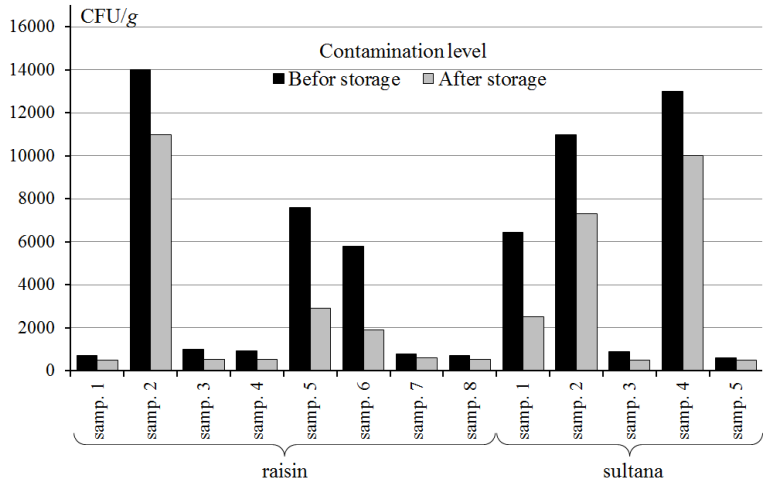


Fig. 3. Contamination level of Armenian black dried vine fruit samples after and before storage at temperature lower then 4°*C*.

Conclusion. For developing methods on prevention of dried vine fruit contamination by filamentous fungi and their toxins, it is necessary to study factors influencing on fungi growth and mycotoxin biosynthesis. Establishment of monitoring on some parameters (temperature, storage condition and duration, condition of transportation and drying, etc.) will help objectively forecast the contamination level of dried product by fungi and mycotoxins [21].

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