in application in biotechnology as a tool to interfere the hydrogen metabolism in these bacteria.

References

1. *Basak N, Jana AK, Das D, Saiki D*. Photofermentative molecular biohydrogen production by purple-non-sulfur (PNS) bacteria in various modes: The present progress and future perspective. Int J Hydrogen Energy 2014;39:6853–71.
2. *Gabrielyan L, Sargsyan H, Hakobyan L, Trchounian A.* Regulation of hydrogen photoproduction in *Rhodobactersphaeroides*batch culture by external oxidizers and reducers. Appl Energy 2014; 131: 20–25.
3. *Gabrielyan L, Trchounian A.* Purple bacteria and cyanobacteria as potential producers of molecular hydrogen: an electrochemical and bioenergetic approach. In: Trchounian A, editor. Bacterial Membranes. Ultrastructure, bioelectrochemsitry, bioenergetics and biophysics, Kerala, India: Research Signpost; 2009, p. 233–273.
4. *Hakobyan L, Gabrielyan L, Trchounian A.* Bio-hydrogen production and the F_oF_1-ATPase activity of *Rhodo bacter sphaeroides*: effects of various heavy metal ions. Int J Hydrogen Energy 2012; 37: 17794–17800.
5. *Hakobyan L, Gabrielyan L, Trchounian A*. Yeast extract as an effective nitrogen source stimulating cell growth and enhancing hydrogen photoproduction by *Rhodo bacter sphaeroides*strains from mineral springs. Int J Hydrogen Energy 2012; 37: 6519–6526.
6. *Han H, Liu B, Yang H, Shen J.* Effect of carbon sources on the photobiological production of hydrogen using *Rhodobactersphaeroides* RV. Int J Hydrogen Energy 2012; 37: 12167–74.
7. *Marone A, Izzo G, Mentuccia L, Massini G, Paganin P, Rosa S, Varrone C, Signorini A.* Vegetable waste as substrate and source of suitable microflora for bio-hydrogen production. Renewable Energy 2014; 68: 6–13.
8. *Rollina JA, del Campoa JM, Myunga S et al*. High-yield hydrogen production from biomass by in vitro metabolic engineering: Mixed sugars co-utilization and kinetic modeling. *Proc Nat. Acad.Sci. USA* 2015; 112: 4964-4969.
9. *Sargsyan H., Gabrielyan L., Trchounian A*. The distillers grains with solubles as a perspective substrate for obtaining biomass and producing bio-hydrogen by *Rhodo bacter sphaeroides.* Biomass & Bioenergy 2016; 90, 90-94.
10. *Trchounian A.* Mechanisms for hydrogen production by different bacteria during mixed-acid and photo-fermentation and perspectives of hydrogen production biotechnology. Crit Rev Biotechnol 2015; 35: 103-13.
11. *Wang LJ.* Production of bioenergy and bioproducts from food processing wastes: a review. Transactions of the ASABE 2013; 56: 217–30.

Biolog. Journal of Armenia, 1 (69), 2017

THE CONTENT OF SULFUR DIOXIDE IN DRIED VINE FRUIT REALISED IN ARMENIA

L.L. HAKOBYAN[1], K.M. GRIGORYAN[2], A.H. TRCHOUNIAN[1,2]

[1] Department of Biochemistry, Microbiology and Biotechnology, Yerevan State University;
[2] Research Institute of Biology, Yerevan State University
hakobyanl@ysu.am

In dried vine fruit production much attention is paid to the development of technologies and methods for inhibition the growth and evolution of potentially toxigenic fungi. The most effective method to limit the fungi growth is the treatment of raw grape with sulfur dioxide. However, the

usage of SO_2 is limited by international sanitary regulations. The purpose of this work is to identify the correlation between sulfur dioxide content and the contamination degree of dried vine fruits by filamentous fungi, as well as to determine if the quantity of sulfur dioxide in dried vine fruits sold in Armenia meets the requirements presented in international sanitary rules and regulations.

Sulfur dioxide – dried vine fruit – grape – filamentous fungi

Չորացրած խաղողի արտադրության մեջ մեծ ուշադրություն է դարձվում պոտենցիալ թունածին միցելիալ սնկերի աճը և զարգացումը արգելակող տեխնոլոգիաներին և մեթոդներին: Սնկերի աճի սահմանափակման ամենաարդյունավետ մեթոդն է հումքային խաղողի մշակումը ծծմբի երկօքսիդով (SO_2): Սակայն SO_2-ի կիրառումը սահմանափակված է միջազգային սանիտարական կանոնակարգերով: Այս աշխատանքի նպատակն է՝ որոշել SO_2-ի պարունակության և միցելիալ սնկերով չորացրած խաղողի աղտոտվածության աստիճանի միջև կապը, ինչպես նաև բացահայտել թե Հայաստանում իրացվող չորացրած խաղողում SO_2-ի պարունակությունը որքանով է համապատասխանում միջազգային սանիտարական կանոներին և նորմերին:

Ծծմբի երկօքսիդ –չորացրած խաղող – խաղող – միցելիալ սնկեր

В производстве сушеного винограда большое внимание уделяется технологиям и методам ингибирования роста и развития потенциально токсигенных грибов. Самым эффективным методом для лимитирования роста грибов является обработка сырьевого винограда диоксидом серы. Однако, использование SO_2 лимитировано международным санитарным законодательством. Целью настоящей работы является выявление корреляции между содержанием диоксида серы и степенью контаминации сушеного винограда мицелиальными грибами; а также определение соответствия международным санитарным правилам и нормам количества диоксида серы в сушеном винограде реализуемом в Армении.

Диоксид серы – сушеный виноград – виноград – мицелиальные грибы

Favorable climatic conditions in countries with well-developed viticulture contribute to the spreading of mold fungi potential producers of ochratoxin A (OTA). As a result of mycological analyzes carried out by many authors in different countries OTA was frequently detected in grapes and grape derived products, especially in different types of dried vine fruits [1, 2, 9, 10]. In order to reduce the risks of contamination of the final product during dried vine fruit production much attention is paid to the development of technologies and methods for inhibition the growth and evolution of mycotoxigenic filamentous fungi. The use of appropriate agrotechnical means and fungicides in grape cultivation can significantly prevent the growth of fungi and contamination of final product by toxigenic species [6]. In post harvest period, the most effective method for limiting the development of mold fungi is the treatment of raw grape with sulfur dioxide [8].

Sulfur dioxide (SO_2) is used in both gas state and powder form of its sulphite, bisulphite and metabisulphite salts. These compounds are considered relatively strong preservatives because of their strong antimicrobial activity. It also acts as an antioxidant that prevents oxidative processes, which results in a discoloration of final product [3]. Some sultana and raisin types of grape are treated with sulfur dioxide for getting a lighter and golden color [7]. Sulfitation of row materials is usually carried out by gas fumigation or immersion in 2-3% sulfuric acid solution. In Armenia dry sulfitation is widely accepted.

However, it was reported that the residual amount of sulfur dioxide can have a dangerous effect on the health of consumer. Food intake with sulfite content, according to Settipane [16], caused acute allergic reactions in humans and anaphylactic shock with a fatal outcome. It is also known about dangerous effect of SO_2 on asthmatic patients [18]. SO_2 and sulfites greatly reduce the absorption of vitamin B1 in human organism, which can lead to a number of health problems, such as chronic headaches and impairment of memory. The residual amount of SO_2 in the grapes and in the products of its processing should not exceed the limit of 1500 mg / kg, established by the Codex Alimentarius [4].

The purpose of this work is to identify the correlation between sulfur dioxide content and the contamination degree of dried vine fruits by filamentous fungi, as well as to determine if the quantity of sulfur dioxide in different varieties of dried vine fruits sold in Armenia meets the requirements presented in international sanitary rules and regulations.

Materials and methods

Mycological analyses of dried vine fruit samples were carried out with direct planting and serial dilution planting methods [12]. The dilution includes following steps: 1) shaking the product suspension for 15 minutes on a shaker; 2) infusion for 10 minutes; 3) preparation of serial dilutions 1:10 and 1: 100; 4) surface plating from dilutions on nutrient mediums. Plates were incubated at 25±1 °C for 7 days. Following nutrient mediums were used: CYA (Chapek-Yeast Agar medium, HiMedia Ltd.), GYA (Glucose-Yeast Agar medium, HiMedia Ltd.), and MEA (Malt-Extract Agar medium, HiMedia Ltd.).

The quantity of microscopic fungi in 1 g food was detected according to NF ISO 7698-91 [5]:

$$\omega = \frac{\sum C}{(n_1 + 0.1\ n_2)d}$$

$\sum C$ –sum of colonies of fungi grown in all dishes; d – dilution coefficient;

n_1 – number of dishes used for I dilution; n_2 – number of dishes used for II dilution.

The quantity of SO_2 in dried vine fruit samples was determined by aspiration method [20]. The method consists of 3 stages:

1. Determination of free SO_2 – involves aspiration a mixture of sample homogenate and 25% phosphoric acid solution, for 15 minutes and titration with 0.01M NaOH solution until an olive green color appears. Registration of title value (A).

2. Determination of bound SO_2 - involves aspiration 15 min and simultaneously heating solution with content of a sample homogenate. Titration of solution with 0.01 M NaOH until an olive green color appears. Registration of title value (B).

3. Calculation

$$\text{Free } SO_2 \text{ (mg/l)} = \frac{(A) \times M \times 32.06 \times 1000}{V} \quad \text{Bound } SO_2 \text{ (mg/l)} = \frac{(B) \times M \times 32.06 \times 1000}{V}$$

Total SO_2 (mg/l) = Free SO_2 (mg/l) + Bound SO_2 (mg/l),

M = molarity of the NaOH solution, V = sample volume

Results and discussion

In recent years the volume of imports of dried vine fruit exceeds the volume of its production in Armenia [17]. Dried grape are imported into Armenia mainly from Turkey, Iran, as well as from other countries: Uzbekistan, Kazakhstan and USA (California). The results showed that the contamination level of Armenian, Uzbek and Kazakh dried vine fruits by filamentous fungi often exceeds the maximum permissible level of this parameter presented in local and international sanitary rules and regulations (table 1). According to SanPin 2.3.2.1078-01 maximum permissible amount of diasporas of molds in 1 g of dried vine fruit is 5 x102 CFU/g.

Table 1. The number of fungi species in genera isolated from dried grape produced in different countries and the contamination level of samples

Genus	The number of species isolated from dried vine fruit samples produced in different countries				
	Armenia	Iran / Turkey*	USA	Uzbekistan	Kazakhstan
Aspergillus	13	6	3	3	3
Penicillium	10	4	2	2	2
Mucor	2	2	1	2	2
Alternaria	1	-	-	-	-
Trichoderma	-	1	-	-	-
Syncephalastrum	1	-	-	-	-
Total number of species	27	13	6	7	7
Contamination level (CFU/g)	$2.2x10^2$ – $4.3x10^4$	$2.3x10^2$ – $4.8x10^2$	$1.8x10^2$ – $3.64x10^2$	$2.7x10^2$ – $5.8x10^3$	$1.82x10^2$ – $1.7x10^3$

* Sometimes it was difficult to find out exactly from which country (Iran or Turkey) the product was imported

In order to identify the correlation between content of sulfur dioxide (SO_2) and contamination level of dried vine fruit by filamentous fungi, the quantities of bound and free sulfur dioxide were determined in 30 samples of dried vine fruits by aspiration method. The high total amount of sulfur dioxide up to 1350 mg/kg was observed in Iranian and Turkish samples (seedless variety) (Fig. 1). The contamination degree of these dried vine fruit samples (sultana) by micromycetes did not exceed the value of $1.9x10^2$ CFU/g. In local produced samples of white varieties of dried vine fruit the content of bound and free sulfur dioxide met the requirements of Codex Alimentarius Commission [4].

At the 30 mg/kg residual amount of sulfur dioxide the contamination level of samples by fungi exceeded the value of $7.4x10^2$ CFU/g. This does not meet the microbiological safety requirements set out in SanPiN 2.3.2.1078-01 [14]. The content of "free" sulfur dioxide (SO_2) in Iranian and Turkish samples was in range of 45 - 80 mg/kg. The inhibitory effect of SO_2 on quantity of viable spores - potential producers of ochratoxin A occurred when the residual amount of free sulfur dioxide was 60 mg/kg. In analyzed samples of Iranian and Turkish dried vine fruit the total content of sulfur dioxide was 900 - 1350 mg/kg, which did not exceed the maximum allowable concentrations. But it was very high and could harm consumer with diseases in upper respiratory tract. The total content of sulfur dioxide in samples of Armenian white dried vine fruit was detected within 600 - 800 mg/kg. Sporulation degree of these samples was in rang of $2.3x10^2$- $7.4x10^2$ CFU/g (Fig. 1).

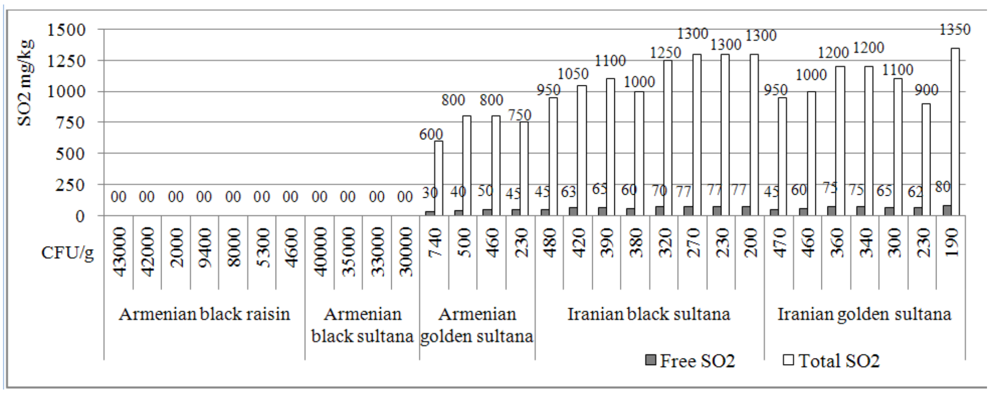

Fig. 1.Correlation between SO_2content in dried vine fruit and their contamination level by filamentous fungi

Dried vine fruit produced from black or dark red grape varieties are not treated with sulfur dioxide, which causes the high contamination degree of black dried vine fruit samples by filamentous fungi. The results of mycological analysis of 11 Armenian black dried vine fruit samples showed high contamination levels of analyzed samples by micromycetes: in range of $4.6x10^3$– $4.3x10^4$ CFU/g.

Sulfur dioxide and its derivatives have long been used for treatment of raw grape as a preservative inhibiting the development of mold-forming fungi and other microorganisms. Previously, SO_2 was included in the list of safe chemicals, for which no control was required. But based on the results of studies that proved its dangerous effect on the human health, today its quantity in food products is limited by the international sanitary rules and norms. For this reason many scientific works are done to find safe biological methods to prevent contamination of food products by filamentous fungi.

References

1. *Battilani P., Giorni P., Bertuzzi T., Formenti S., Pietri A.* Black aspergilli and ochratoxin A in grapes in Italy. Int. J. Food. Microbiol. 111. p. S53–S60. 2006.
2. *Bau M., Bragulat M.R., Abarca M.L., Minguez S., Cabanes F.J.* Ochratoxigenic species from Spanish wine grapes. Int J Food Microbiol. 98. p.125–130. 2005.
3. *Chervin C., Aked J., Crisosto C.H.* Grapes: Post – harvest technology for dried grapes. Crop Post-Harvest: Science and Technology. First Edition. p. 195-197. 2012.
4. Codex Alimentarius Committee. CAC/RS 67-1974. Adopted 1981. Codex Standard for Raisin. Codex Stan. 6. p. 1-5. 1981.
5. Controle de la qualite des produitsalimentaires controle micro biologique. NF ISO 7698-91. Direktivesgenerales pour le denombrement des levuresetmoisissures. 1993.
6. *Covarelli L., Beccari G., Marini A., Tosi L.* A review on the occurrence and control of ochratoxigenic fungal species and ochratoxin A in dehydrated grapes, non fortified dessert wines and dried vine fruit in the Mediterranean area. Food Control. 26. p. 347-356. 2012.
7. *Gowda I.N.D.* Evaluation of certain pre-treatments for raisin making. Journal of Food Science & Technology. Mysore. 37. p. 21–125. 2001.
8. *Hocking A.D.* Xerophilic fungi in intermediate and low moisture foods. Handbook of applied mycology, foods and feed. New York: Marcel Dekker Inc. 3. p. 69-97. 1992.
9. *Leong S.L., Hocking A.D., Pitt J.I., Kazi B.A., Emmett R.W., Scott E.S.* Australian research on ochratoxigenic fungi and ochratoxin A. Int J Food Microbiol 111. Suppl 1. p. S10–S17. 2006.
10. *Magnoli C, Violante M, Combina M, Palacio G., Dalcero A.* Mycoflora and ochratoxin-producing strains of black aspergilli in wine grapes in Argentina. Letters in Applied Microbiology. 37. p. 179-184, 2003.
11. Mannsville. Chemical Products Corp. Chemical: Sulfur Dioxide. NY. p. 199 – 213. 1985.
12. *Pitt J.I., Hocking A.D.* Fungi and Food Spoilage. 2nd Edition. Blackie Academic and Professional. London. 1997.
13. *Sander U.H.F., Rothe U., Kola R.* Sulphur dioxide and Sulphuric. An Introduction to Their Industry Chemistry and Technology, London, p. 151-254, 1984.
14. SanPiN 2.3.2.1078-01. Sanitary-epidemiological rules and regulations. Hygienic requirements for the safety and nutritional value of food, 2001.
15. *Shanmugavelue K.G.* Postharvest handling and marketing of grapes. Viticulture, India. 1989.
16. *Settipane G.A.* Adversereactions to sulfites in drugs and foods. Am. Acad. Dermato. 10. p. 1077-1081. 1984.
17. Statistical Yearbook of Armenia - www.armstate.am
18. *Stevenson D.D., Simon R.A.* Sulfites and asthma. Allergy clin. Immunol. 74. p. 469-472. 1984.
19. *Weil E.D.* Sulfur compounds. Encyclopedia of Chemical Technology. 3rd ed. Vol. 22. New York. p. 107-167, 1983.
20. *Wood R., Foster L., Damant A., Key P.* Analytical methods for food additives. 2004.