Study on resveratrol content in grapes and wine products

Petr E Balanov[1,*], *Irina V* Smotraeva[1], *Malika S* Abdullaeva[1], *Daria A* Volkova[2], *and Olga B* Ivanchenko[3]

[1]Faculty of Biotechnologies (BioTech), ITMO University, 197101 Kronverksky 49, Russia
[2]University of Copenhagen (UCPH), 1017 Nørregade 10, Denmark
[3]Peter the Great St. Petersburg Polytechnic University (SPbPU), 195251 Politekhnicheskaya 29, Russia

Abstract. The current state of the agro-industrial complex and the food industry allows to satisfy the needs of various consumers groups. Among those needs, functional foods enriched with beneficial nutrients are becoming more and more popular. Polyphenols are sometimes used as such additives. The bioflavonoid resveratrol, among the group of polyphenols, has very promising antioxidant, therapeutic, antimicrobial properties. In this regard, studying the potential of its use in the food is highly relevant. Resveratrol is naturally present in the dark grapes. It is localized in the skin of the fruit and passes into the food substance during technological processing. This transition has its own patterns which are outlined in this article. Three varieties of grapes were chosen as the objects of the study: Cabernet Sauvignon, Merlot, Krasnostop. Clarified musts from these grape varieties, concentrated musts and wines were researched. High performance liquid chromatography (HPLC) was utilized as the research method. This method allows to separate liquids of complex composition into components with subsequent identification of the mixture composition. To calibrate the chromatograph, the standard "25 mg European Pharmacopoeia", produced in France, was used as a standard for the pure substance of resveratrol. As the result of the study, it was found that resveratrol is present in the studied samples in various amounts from 4,4 to 7,0 mg / dm^3 in the grape juice, from 6,9 to 12,6 mg/dm^3 in the wine materials, from 12,4 to 21,3 mg / dm^3 in the concentrated juice. These data help establishing the influence of the processing technology of wine and juice concentrates on the resveratrol content. The article also discusses the potential of using concentrated grape juice, rich in resveratrol, to obtain various food products.

1 Introduction

Resveratrol (trans-3,4',5-trihydroxystilbene), a natural polyphenolic non-flavonoid antioxidant, is a phytoalexin widely found plants, including grapes, berries, cocoa, and nuts. It is synthesized by plants as a defense against parasites such as bacteria or fungi.

One of the possible mechanisms of its protective activity is the suppression of inflammatory reactions [1].

Recent studies have shown that stilbenes, in particular trans-resveratrol [2] and its glucoside, have certain health benefits, since they obtain antioxidant, cardioprotective, anticarcinogenic and antitumor properties [3,4].

Significant oncoprotective properties are proven by number of studies that allow to draw the conclusions about the positive effect of resveratrol in the prevention of certain diseases and the pronounced stimulating effect in chemical therapy aimed at suppressing the formation of malignant tumors [5].

The main sources of resveratrol include grapes, wine, peanuts and herbal tea from *Polygonum Cuspidatum* (Japanese knotweed), also known as Itadori tea, which has been used in Japan and China for centuries as a traditional herbal remedy for heart diseases and strokes [6,7].

The concentration of trans isomer as the major form in red wine is usually in the range of 0,1 to 15 mg/l. As the phenolic compound, resveratrol contributes to the antioxidant potential of red wine and thus may play a role in the prevention of human cardiovascular diseases [8,9,10].

The cardioprotective activity of resveratrol is associated with inhibition of platelet aggregation and oxidation of low-density lipoprotein (LDL), followed by increased artery vasorelaxation [11].

Numerous in vitro studies describe the different biological effects of resveratrol. The main effects are antioxidant, anti-inflammatory and estrogenic effects, as well as antitumor and chemopreventive effects [12,13,14,15].

Number of clinical trials have shown that resveratrol might be beneficial for patients with diabetes. The therapeutic effect of this compound on diabetes is complex and includes several beneficial functions [16].

The mechanism by which resveratrol improves insulin action includes reducing obesity, altering gene expression, and altering the activity of certain enzymes. These data indicate that resveratrol may be useful in the prevention and treatment of diabetes. The use of resveratrol, alone or in combination with modern antidiabetic therapies, may become the traditional

* Corresponding author: balanov@yandex.ru

approach to effective treatment of diabetes mellitus or its complications [17,18].

Since resveratrol is a natural antimicrobial agent, the usage of it has become widely spread among researchers for the treatment of acute and chronic inflammatory diseases [19,20].

One of the most popular sources of resveratrol is grapes. The content of resveratrol in the pulp, skin, seeds and stalks of different grape cultivars varies significantly [21,22,23,24].

Due to the important properties of resveratrol, there is a growing interest in the production of products with a higher content of this compound and a higher biological value. The production of grapes and wines with high resveratrol content is based on quality-oriented viticulture (suitable terroirs, sustainable viticultural practices) and wine-making technologies [25].

The authors of this study believe that the use of resveratrol may be justified when creating functional foods. The prerequisites for this assumption are: a strong evidence for the safety of this substance, accumulated data on health benefits, bioavailability, relative ease of isolation and industrial manufacturability when added to food.

The addition of resveratrol to food will most likely play a role in prevention of disease development by providing number of health benefits mentioned above.

The purpose of the study is to follow the dynamics of changes in the concentration of resveratrol in grape raw materials in the following sequence: grapes - concentrated grape juice - wine material - wine.

Additionally, the aim of the work is to research the effect of the duration of grape pulp maceration on the concentration of resveratrol in the wort.

2 Materials and research methods

The study is based on determining the actual content of trans-resveratrol using high performance liquid chromatography (HPLC). This method involves chromatographic studies using a standard of pure resveratrol substance (standard - 25 mg European Pharmacopoeia, France).

The method is based on identification and quantitative calculation of the resveratrol content displayed on chromatograms. The peaks of the chromatograms characterize the presence of the investigated component and the peak area corresponds to its concentration in the medium.

Identification of grape raw material samples was carried out using high pressure liquid chromatography under the following conditions: high pressure liquid chromatograph "Agilent 1200 HPLC-DAD manual" (USA) with isocratic elution mode of the mobile phase, column Supelcosil Express LC20, 250 x 4.6 x 5,0 (mm).

Operating values determined as: wavelength 310 nm and 288 nm. The resveratrol retention time - 90 seconds. The total analysis time for one sample is 24 minutes.

The solvents used: 0.025% trifluoroacetic acid and acetonitrile (ACN) diluted in a 70:30 ratio. The eluent transfer rate was 1 cm^3 / min. The working sample of each sample was 20 µl. Each sample was pre-filtered with a membrane filter. The size of the mesh membrane is 200 µm.

A chromatographic image of resveratrol identified peaks is shown in Fig. 1.

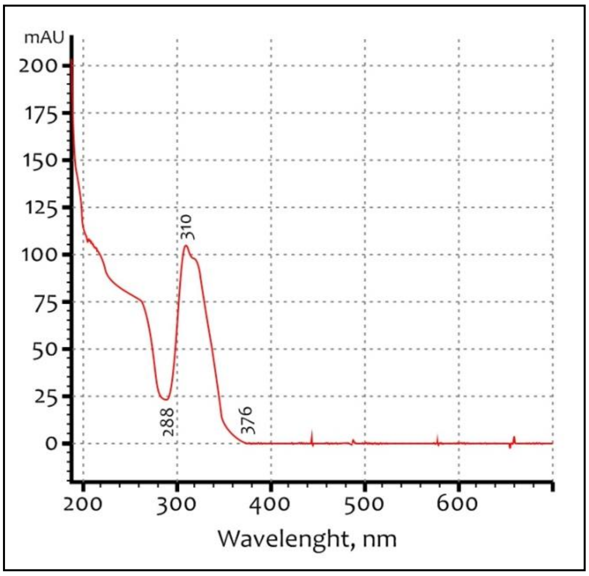

Fig. 1. Chromatographic image of resveratrol received by authors as the result of measurements on an Agilent 1200 HPLC-DAD chromatograph.

Various sources were used as samples:

- fresh juice from grape cultivars: Merlot, Cabernet Sauvignon, Krasnostop
- concentrated red grape must: Merlot, Cabernet Sauvignon, Krasnostop
- wine material of red cultivars: Merlot, Cabernet Sauvignon, Krasnostop
- dry wine of red cultivars: Merlot, Cabernet Sauvignon, Krasnostop

Concentration of fresh juice was carried out to reach 65% of dry substances content and conduct subsequent measurement of resveratrol content in the resulting concentrate. Moisture was removed on a RE3000E rotary vacuum evaporator (China) at the rotation speed of 60 rpm and the temperature of 70^{0}C.

The use of evaporation in a vacuum environment avoids overheating of the solution and prevents oxidation processes.

The method of grape pulp and juice infusion at the temperature of 20^{0}C for two days was utilized to establish the dynamics of resveratrol accumulation when obtaining wine material. Resveratrol content was measured every 8 hours.

Implementing this process allows to create a more intense contact between the skin of the grapes and the juice. As the result, triggered by the action of the solvent and native grape enzymes the plant tissue of the cell membranes becomes thinner. Hence, the diffusion of components from the solid mass is facilitated and the product is enriched with soluble substances.

Method of preservation with the addition of sulfur dioxide was utilized to prevent spontaneous action of microorganisms in the macerated environment. The preservative concentration is 40 mg SO_2 per dm^3 of processed material. This concentration does not exceed existing requirements for the product in wine industry.

The dosage of component was performed by addition of potassium metabisulfite $K_2S_2O_5$ to the medium. It is registered as an approved additive (E224) to be used in the food industry by EU.

3 Results and discussion

The results of HPLC chromatographic study among samples of grape raw materials of various cultivars for the content of trans-resveratrol in them are presented in Table 1.

Analysis of the data obtained allows to conclude that the greatest amount of resveratrol is contained in the Krasnostop grapes. The amount in comparison to Merlot and Cabernet Sauvignon is higher by 37,1% and 10,0%, respectively.

Table 1. Resveratrol content in red grapes.

№	Name of the sample	Resveratrol content, mg/dm³
1	fresh grape juice, Merlot	4.4
2	fresh grape juice, Cabernet Sauvignon	6.3
3	fresh grape juice, Krasnostop	7.0
4	concentrated red grape must, Merlot	12.4
5	concentrated red grape must, Cabernet Sauvignon	18.7
6	concentrated red grape must, Krasnostop	21.3
7	red wine material, Merlot	6.9
8	red wine material, Cabernet Sauvignon	10.9
9	red wine material, Krasnostop	12.6
10	dry red wine, Merlot	6.4
11	dry red wine, Cabernet Sauvignon	9.1
12	dry red wine, Krasnostop	10.1

As the result of juice concentration, the concentration of resveratrol increased 2,8 times in the concentrate from Merlot grape juice, 2,9 times in the concentrate from Cabernet Sauvignon, and 3.0 times in the concentrate from Krasnostop.

It should be noted that the concentration of dry substances in the concentrated juice reached 65%, hence, the increase in the concentration of basic components is 3,1-3,3 times. These data suggest that when the juice was concentrated at the temperatures of 60-70°C, there was no significant decrease in the concentration of resveratrol due to any chemical reactions.

The data obtained implies that there is a relation between the initial content of resveratrol in the treated medium and the accumulation of it in the final product, the relation being directly proportional. That is, the more the researched component was initially found, the more it accumulates in the concentrate.

It could be concluded that the use of concentrated juices will allow to enrich food products with resveratrol without significant losses in the amount and, in the long term, without losses in its bioavailability.

During preparation of the wine material, the infusion of must and wort was implemented under the conditions specified in the section "Materials and methods". Resveratrol concentration was increasing in comparison to directly squeezed juice due to a longer contact between the skin and juice. The increase in the resveratrol concentration was 36,2% for the Merlot grapes, 42,2% for the Cabernet Sauvignon grapes, 44,4% for the Krasnostop grapes. The data indicate the significant effect of maceration on the concentration of resveratrol in the medium.

During the wine production wine from these wine materials, a decrease in the concentration of resveratrol was observed. The decrease in the concentration of the component was as follows: by 7,2% for wine made from Merlot grapes, by 19,8% for wine made from Cabernet Sauvignon grapes, by 17,5% for wine made from Krasnostop grapes. This decrease is probably associated with the loss of resveratrol during the technological processing of wine materials - treatment with clarifiers and removal of the formed sediments. In this regard, it should be noted that technological processing of wine materials significantly affects the loss of resveratrol. It could be assumed that this effect is present due to the chemical interaction of polyphenols (including resveratrol) with the components of clarifiers. It seems relevant to study this problem more detailly in the future.

Question of particular interest is the dynamics of resveratrol accumulation during the infusion of grapes on the must. This interest is originated from the fact that the infusion of wort with pulp is a technological method that is frequently used in winemaking. Manufacturers are interested in the total complete transfer of valuable components from pulp to wort in a short period. Thus, the duration of maceration is an important limiting factor for industrial production. The accumulation of this component occurs unevenly over time, it is shown in Fig. 2.

From the data obtained, it is concluded that the longer the duration of the contact between the must and juice, the more resveratrol passes into solution. This system reaches its maximum concentration values after reaching 32 hours. Further growth is negligible.

It should also be noted that the grape variety, the pulp of which is macerated, is essential for the accumulation of resveratrol. At the initial concentration of resveratrol in the varieties Merlot, Cabernet Sauvignon and Krasnostop of 4,4 mg/dm³, 6,3 mg/dm³ and 7,0 mg/ dm³, the increase in concentration was 2,5 mg/dm³, 4,6 mg/dm³ and 5,6 mg/dm³. That is 36%, 73% and 80% of the total increase, respectively. Hence, the higher the initial concentration of resveratrol in grapes, the higher increase in the must concentration one can expect.

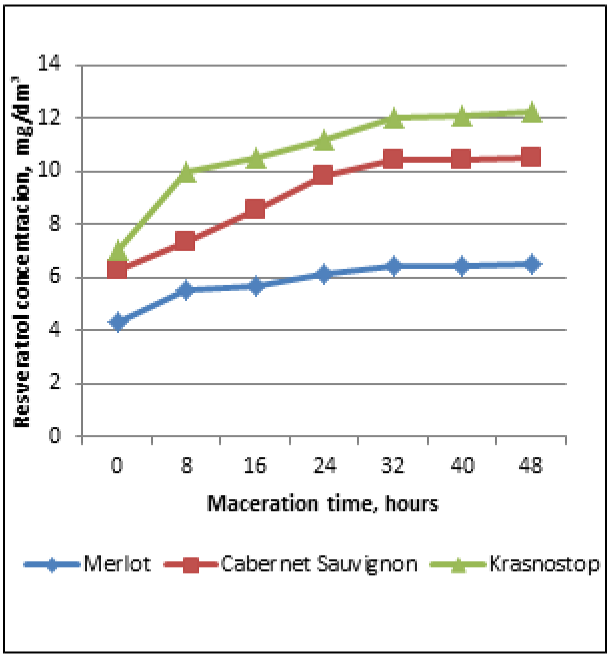

Fig. 2. Dynamics of resveratrol accumulation in the must-juice system.

4 Conclusions

Based on the data, it could be concluded that the behavior of resveratrol in complex environments is predictable.

The data are in good agreement with the results received by other researchers [17].

The use of grape juice concentrate for food fortification is promising.

There are certain prerequisites for the use of specific techniques (for example, infusion), adopted in the technology of winemaking, to obtain concentrated juices with a higher content of resveratrol as compared to traditional methods.

The authors consider the following products as potential ones for the enrichment:

- Confectionery. For instance, fillers for marmalade, since the grape juice concentrate, in addition to resveratrol, contains native sugar which is required by the recipe.

- Soft drinks. The addition of resveratrol-rich grape juice concentrate will establish a functional product status that will expand the consumers base.

- Milk-based products - yoghurts, ice cream. It will expand the range and enhance the functional properties due to the synergistic effect of the probiotic component.

- Products based on animal raw materials. Concentrated grape juice is occasionally used as an ingredient in the meat products industry. The presence of significant amounts of resveratrol in the meat products could serve as a potential incentive for more effective promotion of these products.

For future research, it represents a certain interest to find solutions to replace sulfur dioxide, which is used as a traditional preservative in the wine industry, with components that will not cause certain doubts among the consumers. Native plant components, e.g., benzoic acid of wild berries (cranberries, lingonberries), could be considered as such preservatives.

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